Food security and holistic human development through sustainable aquaculture

2014 SEAFDEC/AQD Highlights

SEAFDEC

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Front cover photo of Igang Marine Station and photos of commodities on inside cover by I Tendencia

Message of the Chief

Time flies so fast. Last year, we celebrated our 41st anniversary and continued implementing our mandate of generating science-based aquaculture technologies for our stakeholders in the country and in the region. Our theme in last year's celebration of "sharing and generating sustainable aquaculture technologies for a prosperous ASEAN community" was indeed appropriate. By December of this year, 2015, ASEAN member countries will start implementing some aspects of the ASEAN Economic Integration (AEI) that, among others, will allow the free trade and flow of goods, including fishery products between and among ASEAN member countries.

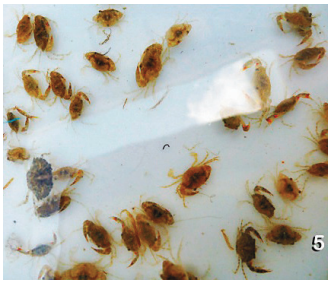
For our stakeholders, this AEI can be viewed from two sides. This provides the challenge and the opportunity to innovate some more in order to improve our production efficiency thereby lowering production costs and making our products more competitive. The other side is the developing fear of losing in the competition. Sadly, this is what I heard from some fish farmers in Davao in one of the consultations I attended early last year. They cited some factors that they feel put them at a disadvantage with the fish farmers from our neighboring countries when AEI happens by the end of this year - high production costs that include high costs of energy, feeds, labor, transport, and other inputs, among others. The fish farmers also perceive the lack of, if not a weak government support to the aquaculture industry.

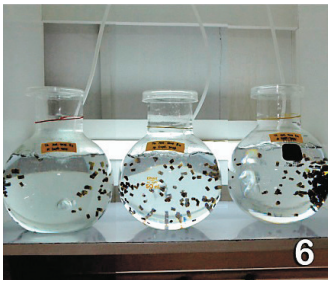
What has AQD done and how can AQD help our fish farmers in this regard? We can help improve production efficiency in many ways thereby lowering production costs. The various studies that we conduct at AQD are geared towards this direction. In the middle of 2014, there was a newspaper report that farmers in northern Luzon were able to produce 500 g milkfish in 5 months using a new feed formulation. It was not mentioned however, that the feed formulation used was actually a product of research in AQD, by Dr. Relicardo Coloso with funding from the United Soybean Board. We are very happy to learn that our research results are now applied in the industry. But we do not stop here. We continue to refine what we feel can still be improved further. As reported by Dr. Coloso, we now have an improved feed formulation for the grow-out culture of milkfish where 500 g fish can be attained in just 3.5 months. While the fish meal content of the feed that was reported in the newspaper was still 50%, in the most recent feed formulation that AQD developed, the fish meal content is now down to 15%. We have tested this in pond and cage conditions. What is yet to be done here is the field testing in larger cages. With this new development, a milkfish grow-out feed that contains no fish meal is probably within reach. We thank Dr. Coloso and our team of nutrition experts for this highly significant achievement.

AQD's scientist, Dr. Emilia Quinitio was in the cover of the June 2014 issue of Agriculture magazine for their feature story on soft-shell mud crab production. Dr. Quinitio led the team that started mud crab R&D in AQD in earnest in 1997. With funding support from ACIAR, the EU, and the Government of Japan Trust Fund, Team mud crab made significant strides in improving the breeding, seed production and culture technologies of the species. Together with our nutrition team, an improved artificial feed for mud crab is also available now. At present, continued improvements as well as nationwide dissemination of production technologies for mud crabs are made possible by the generous financial support that AQD received from DOST/PCAARRD through the National R&D program for mud crab.

We are also eagerly waiting for new developments from our abalone team. Again, our team of nutrition experts headed by Dr. Myrna Teruel are now testing in larger scale the performance of a microparticulate feed for the early stage of abalone production, as well as the grow-out and broodstock feeds for better abalone reproductive performance. These developments are important because these will pave the way for the production of abalone even in areas where supply of seaweed *Gracilaria* is limiting. *Gracilaria,* locally known as gulaman, is a favorite natural food for abalone.

Seaweed is a very important Philippine product. We used to be the top seaweed producer but we have now been overtaken by Indonesia. When super typhoon Haiyan struck, among those that were hit the hardest were the seaweed farmers. They did not only lose their crops, they were also left with no planting materials to start the next crop. AQD has "new" plantlets of *Kappaphycus* produced in the laboratory through tissue culture and these are currently being field tested in the seaweed farms in Bohol, Zamboanga, Pagadian, Batangas, Guimaras and Caluya, Antique. These plantlets are performing very well with growth rates of 6-7% per day compared to the 2 % growth rates of seedlings from cuttings that the farmers have been using for many years. The Seaweed Industry Association of the Philippines, through its Chairman of the Board, Mr. Maximo Ricohermoso, was impressed by the performance of the AQD plantlets that there is now a request for AQD to produce planting materials for a particular strain of *Eucheuma* called "milyon-milyon" because this strain is also very important to the industry. The hard work of our seaweeds group, headed by Ma. Rovilla Luhan and also Dr. Anicia Hurtado during the early years, has paid off. These developments came after many years of testing and refinement. Now that we have the techniques established, we are confident that applying these to other important species will be much easier. Many more of these positive developments are coming from the different laboratories here in the Department.





Harvest of milkfish fed SEAFDEC premium diet (1); an adult abalone (2); feeding microparticulate diet (MPD) to post-larval abalone (3); Dr. E..T. Quinitio on the cover of Agriculture Magazine (4); hatchery-produced crab juveniles (5); culture of seaweed micropropagule in the lab (6); and AQD's junior staff (7)

AQD has been able to achieve all of these because of its most important resources, its human resources – our highly competent and dedicated scientists and support personnel. For AQD to be able to continue providing timely support to its stakeholders, we need to always have people with the same zeal for excellence and dedication. Our scientists have made AQD famous worldwide and we intend to maintain the quality of the scientists that we have. We have recruited 13 new Technical Assistants (TA) to help us implement projects we are undertaking with the different national programs of DOST-PCAARRD. These new TAs are either graduates with honors or they belong to the top 10% of the class from the top universities in the country. These are in addition to the existing 25 TAs already in AQD, majority of them are already in the graduate school. AQD's future pool of experts will come from these very competent young men and women.

The significant achievements we had for the various commodities did not happen overnight. These are products of long and sustained efforts from all of us in AQD to see to it that our target goal for the specific commodity is achieved. Budget has always been a constraint for AQD, but not a hindrance to pursuing excellence. In most of our R&D initiatives, earlier achievements help pave the way for donor support. Aside from the colorful "funding history" of our mud crab R&D, another example is our seaweeds R&D. Initial funding for the tissue culture work came from AQD in the late 90s. The DA-Biotech Program then provided some funds in the early 2000s. The Government of Japan – Trust Fund (GOJ-TF4) provided funds for the refinement of the tissue culture techniques and mass production of plantlets in tanks. The current phase on the establishment of sea-based nurseries and farm trials of the plantlets produced from the laboratory is supported by ACIAR. This situation is also true with the other commodities like milkfish, shrimps, abalone and the others. The continued funding support from our collaborators and funding agencies is very critical for the success of our activities for this allowed us to hire additional personnel, to acquire important laboratory equipment, and helped us accelerate the discovery process. We thank all the funding agencies that collaborated with us through all these years.

Looking back at what AQD has done during the last 41 years, it makes me very proud to see AQD's mark and influence in the aquaculture industry in the country and in the region, which is very tangible and is very much visible. We will stay on our course, as we strive for continued relevance as the provider of technologies that will help fish farmers remain competitive in the era of free trade. We are confident that together, we can do this with all the support that we will get from the national government, the governments of the SEAFDEC member countries, the various donor agencies, and other partners in the aquaculture industry.

Felix G. Ayson, D. Sc.
Chief, SEAFDEC/AQD

New collaborations in 2014

ACADEME

(1) **Ateneo De Manila University (ADMU)** (Loyola Heights, Quezon City): Development of Shrimp Pathogen Diagnostic Tools using nested PCR and lateral flow strip biosensors coupled with a mobile app and cloud-based information management, (01 January 2014 to 31 December 2015)

(2) **Southern Luzon State University** (Lucban, Quezon): Practicum and On the Job Training on freshwater prawn hatchery and grow-out culture, aquaculture genetics; ecology; nutrition and feed development (April 2014 to May 2016)

(3) **Western Philippines University** (Palawan): Conduct of special internship on "Abalone Culture" (24 April-3 May '14)

(4) **West Visayas State University** (La Paz, Iloilo City): Student Internship (May 2014 to June 2014)

(5) **St. Paul University of Iloilo** (General Luna St. Iloilo City): Collaboration on instruction, research, training and extension programs in fisheries, aquatic and related sciences (30 July 2014 to 29 July 2019)

(6) **Iloilo State College of Fisheries** (Tiwi, Barotac Nuevo, Iloilo): Co-management of aquaculture research ponds for *Penaeus monodon* and tilapia. Develop pond management strategies to prevent and control shrimp diseases through ecological approaches and test the designed scheme in large commercial ponds (August 2014 to April 2015)

(7) **University of San Agustin, Inc.** (General Luna St. Iloilo City): Collaboration on instruction, research, training and extension programs in fisheries, aquatic and related sciences (15 September 2014 to 14 September 2017)

(8) **Ateneo De Manila University** (Loyola Heights 1108, Quezon City): Academic and Research Cooperation between the two institutions (14 October 2014 to 13 October 2017)

(9) **West Visayas State University** (Lapaz, Iloilo City): Collaboration on instruction, research, training and extension programs in fisheries, aquatic and related sciences (17 October 2014 to 16 October 2019)

(10) **Bohol Island State University** (San Isidro, Calape, Bohol): Resource Enhancement Project on Napoleon Wrasse *Cheilinus undulatus* (18 November 2014 to 18 November 2015).

GOVERNMENT & NON-GOVERNMENT ORGANIZATIONS

(11) **DOST- PCAARRD (Department of Science and Technology – Philippine Council for Agriculture, Aquatic & Natural Resources Research & Development)** (Los Banos, Laguna): Philippine Shrimp Pathogenomics Program: Development of Diagnostic Tools through Genomics and Establishment of the Philippine Shrimp Pathogen Bio-Bank and Online Biosurveillance Information Resource (3rd month after receipt of funds 2014 to 2016).

(12) **DOST- PCAARRD – University of the Philippines Visayas** (Los Banos, Laguna: Miagao, Iloilo): National R&D Program for Oyster, Program A. Development of the Broodstock and Hatchery Technologies for the Tropical Oyster, *Crassostrea iredalei*, in the Philippines (2 January 2014 to 01 January 2017).

(13) **DOST- PCAARRD - Samar State University**, (Los Banos, Laguna and Catbalogan, Samar): National R&D Program for Oyster, Program B. Increasing Production and Improving quality of oysters *Crassostrea iredale*i, produced in the Philippines (2 January 2014 to 01 January 2017)

(14) **Zoological Society of London-Community, Philippines and Foundation for the Philippine Environment (FPE)** (48 Burgos St., Lapaz, Iloilo City and 77 Matahimik St., Teachers Village, Quezon City): Conduct training course on Mangrove Conservation, Management and Rehabilitation (10 March 2014 to 14 March 2014)

(15) **National Fisheries Research and Development Institute** (Quezon City, Metro Manila): Conduct of a training course on "Capacity Building on Information Dissemination & Data Management" (10-18 March 2014)

(16) **Department of Science and Technology** (Philippines): Conduct of specialized training course on "Mud crab Hatchery & Nursery Operations" (28 April-16 May, 12 May-18 June, 17 May-5 June, 22 May-18 June, 27 May-18 June 2014)

(17) **Japanese Society of Fish Pathology (JSFP)** (Japan): Make plans with JSFP in order to achieve an effective scientific cooperation including scientific seminars, (May 2014 to May 2019)

(18) **Local Government of the Municipality of New Washington** (Brgy. Pinmuk-an, New Washington, Aklan): Grow-out culture of oyster (*Crassostrea iredalei*) using different methods (30 May 2014 to 29 May 2015)

(19) **Japan International Research Center for Agricultural Sciences (JIRCAS)**, Japan, Advancing integrated multi-trophic aquaculture in Milkfish Mariculture in the Philippines: Exante Analysis Using Sustainable Livelihood Approach (7 May 2014 to 27 February 2015)

(20) **Japan International Research Center for Agricultural Sciences (JIRCAS)** (Japan): Integrated multi-trophic aquaculture (MTA) field using fish, seaweeds, oyster and sandfish in marine pens (7 May 2014 to 27 February 2015)

(21) **PCAARRD** (Los Banos, Laguna): Integrated and Sustainable Development Program for the Shrimp Industry (19 September 2014 to 18 September 2017).

PRIVATE SECTOR WITH GOVERNMENT ORGANIZATIONS

(22) **Engr. Reynaldo Tenedero** (ICDC Construction and Supply, Jaro, Iloilo City): Construction of a Commercial-Scale Model Mud Crab Hatchery in the Province of Bohol, DOST-PCAARRD mud crab project (24 January 2014 to 23 July 2014)

(23) **Mr. Mario Santos (Marial Hatchery)**, Dalahican, Quezon, Mudcrab Hatchery and Nursery Operation, DOST-PCAARRD mud crab project. (10 April 2014 to 9 April 2015)

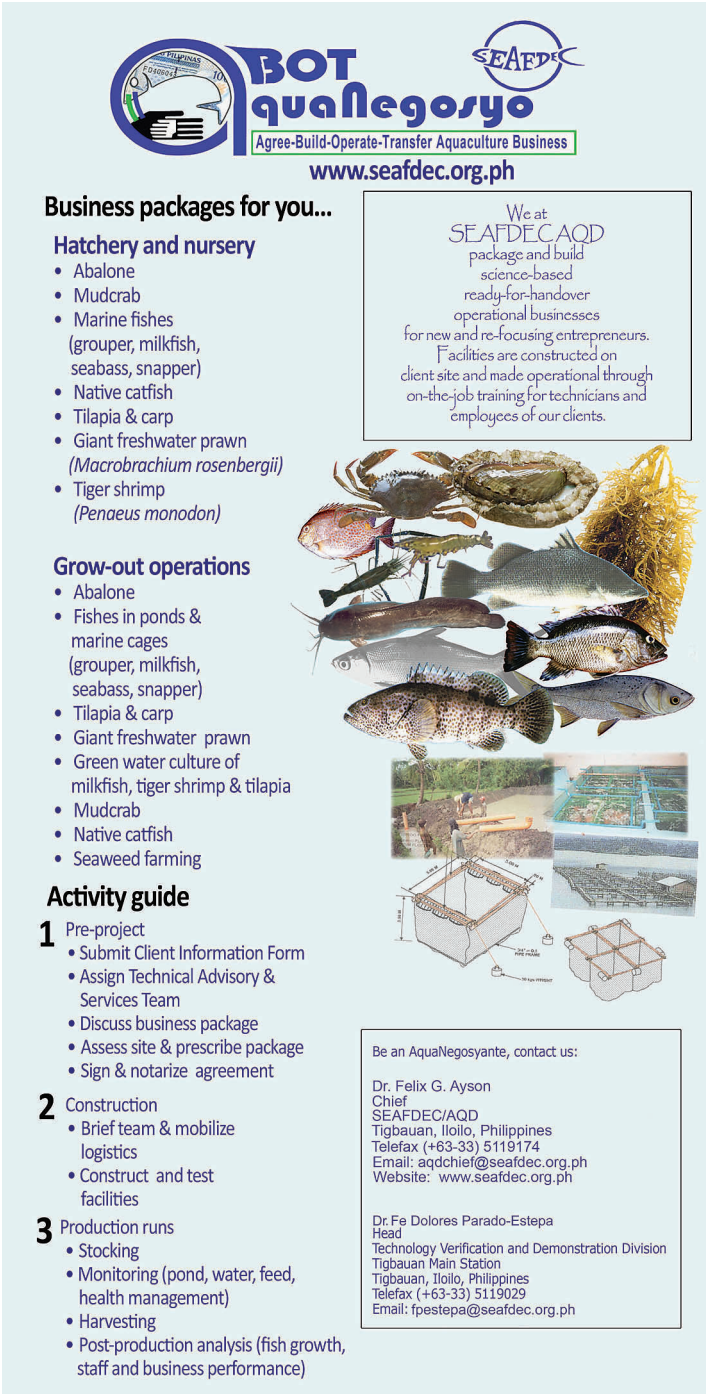
(24) **Mrs. Johaida R. Buenafe (Sipalay Agri-Ecotourism Corp.)** (2011 Tower 2, Pioneer Highlands, Madison St. Mandaluyong City): Provide technical advice and co-supervision with the client in the construction & test of aquaculture facilities. DOST-PCAARRD mud crab project. (08 May 2014 to 07 May 2015)

(25) **Ms. Gretchen M. Torno** (1217 Acacia Street, Dasmarinas Village, Makati City): Provide technical advice and co-supervision with the client in the construction & test of aquaculture facilities, DOST-PCAARRD mud crab project. (05 May 2014 to 04 May 2015)

(26) **Palawan Aquaculture Corporation** (Baquid, Coron, Palawan): Mudcrab Hatchery and Nursery, DOST-PCAARRD mud crab project. (19 September 2014 to 18 September 2015)

(27) **Gretchen Montelibano-Torno** (EB Magalona, Negros Occidental): Mudcrab Hatchery and Nursery, DOST-PCAARRD mud crab project. (19 September 2014 to 18 September 2015)

The ABOT AquaNegosyo
Program of SEAFDEC/AQD



AQD Chief Dr. Felix Ayson (2nd from left) and SPU president Sr. Carolina Agravante sign the agreement with (L-R) AQD Administration and Finance Division head Ms. Kaylin Corre, Research Division head Dr. Ma. Junemie Hazel Lebata-Ramos, Deputy Chief Dr. Takuro Shibuno and SPU Research Director Ms. Imelda Olaguer as witnesses.

Research and Development Programs in 2014

	Study Title	Proponents	Collaborating Partner/s*
THEMATIC PROGRAMS			
Quality Seed for Sustainable Aquaculture (QSSA)			
1	Refinement of sandfish hatchery techniques	FG Ayson/ MJHL Ramos/ JP Altamirano	ACIAR[1]
2	Genetics for sustainable milkfish aquaculture. I. Development and application of molecular markers in milkfish broodstock management	MR Eguia	DOST[2]/UP[3] Institute of Biology
3	Refinement of mudcrab hatchery technology: Project Title 2. Improvement of larval rearing protocol	ET Quinitio	DOST-PCAARRD[4]
4	Development of techniques for sustainable production of good quality captive *Penaeus monodon* broodstock and spawners and high health fry	FDP Estepa	DOST/UPV[5]
5	Refinement of abalone culture techniques to enhance growth, meat quality and maturation: I. Experimental hybridization of Philippine native abalone species; *Haliotis asinina*, *H. glabra*, *H. ovina*, *H. varia*, *H. planata*; II. Triploid induction *Haliotis asinina*	MR de la Peña	DOST-PCAARRD
6	Hatchery and nursery operation for blue swimming crab, *Portunus pelagicus*	FDP Estepa	DOST-PCAARRD
7	Studies on seed production and release of seahorses for stock enhancement *(PhD Dissertation)*	SMB Ursua	JSPS[6]-Ronpaku
8	Improvement and larval rearing of *Portunus pelagicus*	ET Quinitio	DOST-PCAARRD
9	Improved larval rearing protocol for silver therapon (*Leiopotherapon plumbeus*): II. Larval diets, optimal stocking density and weaning strategies under controlled conditions	FA Aya	
10	Application of strategies to reduce cannibalism in the mud crab nursery	FDP Estepa	DOST-PCAARRD
11	Refinement of hatchery techniques for the donkey's ear abalone *Haliotis asinina:* I. Improvement of fecundity and seed quality of breeders recently acquired from the wild; II. Evaluation of genetic stocks for selective breeding of abalone; III. Increase survival rate of veliger larvae through improvement of harvest and incubation protocol	MR de la Peña	DOST-PCAARRD
12	Use of *Cocconeis* sp. *Nitzchia* sp. and *Diploneis* sp. as alternative benthic diatom feed species in the hatchery rearing of abalone *Haliotis asinina*	MR de la Peña	DOST-PCAARRD
13	Refinement of seed production techniques for high value marine fish species such as grouper, red snapper, seabass, rabbit fish and pompano: Metamorphosis and survival of high value marine fish larvae fed sodium iodide-enriched rotifer and *Artemia*	OS Reyes	
14	Development of maturation diet for grouper (*Epinephelus fuscoguttatus*) broodstock: Use of paprika as natural pigment in grouper maturation diet and its effect on egg quality and larvae	OS Reyes	
15	Development of techniques for sustainable production of marine annelids as feed for mud crab broodstock	VR Alava	DOST-PCAARRD
16	Effect of tryptophan-supplemented diets, stocking density and feeding management on survival and growth of mud crab *Scylla serrata* in the nursery phase	VR Alava	DOST-PCAARRD
17	Production of juvenile and marketable size tropical abalone *Haliotis asinin*a in tanks: I. Microparticulate diet as alternative feed in abalone hatchery production: Comparative evaluation in small- and large-scale tank systems	MB Teruel	DOST-PCAARRD
18	Nursery rearing techniques for seed production of *Kappaphycus* "seedlings"	MRJ Luhan	
19	Demonstration on the effect of feeding fortified diet to milkfish broodstock on egg and fry quality	OS Reyes	
20	Broodstock management and conditioning for the oyster *Crassostrea iredalei* to maximize production	JM Ladja	DOST-PCAARRD

Study Title		Proponents	Collaborating Partner/s*
21	Refinement of hatchery and nursery rearing techniques for the production of quality oyster *Crassostrea iredalei*: I. Optimization of induced spawning method II. Improvement of culture techniques.	JM Ladja	DOST-PCAARRD
22	Refinement of mudcrab hatchery technology: Project Title A. Promotion of mud crab hatchery and nursery technologies and B. Pilot demonstration for a commercial scale model mud crab hatchery	ET Quinitio	DOST-PCAARRD
23	Mass production of sex-reversed and mixed Nile tilapia (*Oreochromis niloticus*), and hybrid red tilapia fingerlings	DM Reyes Jr.	
24	Large-scale production of donkey's ear abalone, *Haliotis asinina* juveniles	D Catedral / NC Bayona	
Healthy and Wholesome Aquaculture (HWA)			
25	Improvement of feed formulation for milkfish (*Chanos chanos*) culture in ponds and cages	RM Coloso	DOST/UPV
26	Evaluation of milkfish by-product hydrolysate as ingredient in juvenile grouper, *Epinephelus coioides* diets.	RE Mamauag	
27	Distillers dried grains with solubles (DDGS) evaluation as protein ingredient for milkfish, *Chanos chanos* diets.	RE Mamauag	
28	Improvement of feeds and management practices for mud crab grow-out culture: Pilot-scale production of pellets suitable for mud crab	RM Coloso	DOST-PCAARRD
29	Feed development for the golden pompano, *Trachinotus blochii*.	MR Catacutan	
30	Evaluation of existing feed formulations for mud crab grow-out	MR Catacutan	DOST-PCAARRD
31	Use of soybean meal and soy protein concentrate as alternatives to fish meal in practical feeds for milkfish, *Chanos chanos*	RM Coloso	USB[7]
32	Improvement of the nutritional value of locally available feed resources for practical aquatic feeds by submerged fermentation and solid substrate fermentation using milkfish gut bacteria and/or selected fungi	RM Coloso	
33	Establishment of Philippine shrimp pathogen bio-bank and online biosurveillance resource	EC Amar	DOST-PCAARRD
34	Establishment of sanitary quality of oysters and their culture environments	RV Pakingking Jr.	DOST-PCAARRD
35	Development of shrimp pathogen diagnostic tools using nested PCR and lateral flow strip biosensors coupled with a mobile app and cloud-based information management	LD de la Peña	DOST-PCAARRD
36	Quantitative and qualitative analyses of the bacterial microbiota of tilapia (*Oreochromis niloticus*) cultured in earthen ponds as tool for investigating emerging and re-emerging diseases of tilapia in the Philippines	RV Pakingking Jr.	DOST-NRCP[8]
37	Surveillance of emerging diseases in wild and farmed mud crab: Application of conventional and molecular approaches	EA Tendencia	DOST-PCAARRD
38	Novel strategies to reduce disease incidence in mud crab hatchery and grow-out	EC Amar	DOST-PCAARRD
39	The application and mode of action of probiotic *Bacillus* species in the larviculture of *Penaeus*: Substudy (2) Identification, isolation and characterization of AHL-degrading *Bacillus* species for use as probiotics in *Penaeus monodon* culture *(PhD Dissertation)*	JLQ Laranja Jr.	IFS[9]
40	Application and mode of action of polyhydroxybutyric acid (PHB) in the larviculture of *Penaeus monodon (PhD Dissertation)*	GL Ludevese-Pascual	VLIR-UOS[10]
41	Production characteristics of the giant freshwater prawn *Macrobrachium rosenbergii* cultured in cages using different grow-out management strategies	MLC Aralar	
42	Production of juvenile and marketable size tropical abalone *Haliotis asinina* in tanks: II. Test of refined formulated feed for the grow-out culture of tropical abalone, *Haliotis asinina* Linne in land-based tanks: Use of binders and application of different feed forms.	MB Teruel	
43	Performance of hatcher-bred *Penaeus indicus* in grow out.	SS Avanceña	

Study Title		Proponents	Collaborating Partner/s*
44	Nursery culture of pompano (*Trachinotus blochii*) in floating net cages using formulated feeds	EDJ Ayson	
45	Grow-out culture of pompano (*Trachinotus blochii*) in floating net cages using formulated feeds	EDJ Ayson	
46	Culture of *Gracilariopsis heteroclada* Zhang & Xia in pond and in intertidal area	SS Avanceña	
47	Production of rabbitfish (*Siganus guttatus*) in semi-intensive grow-out culture using formulated diet in brackishwater ponds	EB Coniza	
48	Demonstration of semi-intensive grow-out culture of grouper (*E. fuscoguttatus/ coioides*) fed SEAFDEC/AQD phased-diet in ponds	MR Catacutan	
49	Refinement of the Mud Crab Nursery Technology Project Title: 3. Development of' protocol for the production of hatchery-reared mud crab *Scylla serrata* for the soft-shell crab farming	ET Quinitio (RPCB Ragus)	DOST-PCAARRD
50	Development of ice-ice resistant strains of *Kappaphycus* and technology for reduction of epiphytes	MRJ Luhan	ACIAR
Maintaining Environmental Integrity Through Responsible Aquaculture (MEITRA)			
51	Refinement of abalone grow-out culture techniques in marine environment	MJHL Ramos	DOST-PCAARRD
52	Refinement of existing oyster grow-out techniques	MJHL Ramos	DOST-PCAARRD
53	Biodiversity in the milkfish and shrimp fry (*semilya*) fishery in the surf zones in southern and western Panay	TU Bagarinao	
54	Marine biodiversity around the SEAFDEC Aquaculture Department in Tigbauan, Iloilo	TU Bagarinao	
55	Impact of aquaculture in a freshwater environment: Biodiversity of aquatic fauna at the east and west cove of the Binangonan Freshwater Station	MLC Aralar	
56	Polyculture of sea cucumber with selected marine fish species	JM Zarate	WorldFish Center[11]
57	Hydrographic profiling of Igang Marine Station	JP Altamirano	
58	Culture trial for sandfish *Holothuria scabra* in ponds and sea ranch	JP Altamirano	ACIAR
Adapting to Climate Change (CC)			
59	Effect of elevated temperature and acidity on growth, early development and reproduction of abalone (*Haliotis asinina*)	FL Pedroso	
Meeting Social and Economic Challenges in Aquaculture (MSECAP)			
60	Demonstration and adoption of cage culture of giant freshwater prawn (GFP) *Macrobrachium rosenbergii* among smallholder fish farmers in Laguna de Bay	EV Aralar	
61	Coastal area capability development through community-based stock enhancement in New Washington, Aklan, Philippines	JP Altamirano	RIHN[12]
GOJ Trust Fund Program - The Promotion of Sustainable Aquaculture and Resource Enhancement in Southeast Asia (ASEAN-SEAFDEC FCG Mechanism)			
Promotion of Sustainable and Region-Oriented Aquaculture Practices			
62	Genetic improvement in the giant freshwater prawn, *Macrobrachium rosenbergi*i (III) Assessment of effective broodstock management schemes for improved growth and reproductive performance using two geographically distinct stocks	MRR Eguia	GOJ-TF[13]
63	Development of hatchery techniques for pompano and spotted scat	FL Pedroso	GOJ-TF
64	Development of efficient and low pollution feeds for grow-out and broodstock (freshwater prawn, milkfish, grouper, mud crab, and black tiger shrimp) I. Partial replacement of fishmeal with cowpea meal in practical diets for giant freshwater prawn *Macrobrachium rosenbergii*	FA Aya	GOJ-TF
65	Establishment of management technology for disease tolerance and sustainable aquaculture environment	EA Tendencia	GOJ-TF/RESCOPAR[14] Wageningen University
Food Safety of Aquaculture Products in Southeast Asia			
66	Withdrawal period of antibiotics in fish species cultured in the tropics: Pompano *(Trachinotus blochii)*	EA Tendencia	GOJ-TF

Study Title		Proponents	Collaborating Partner/s*
67	Surveillance of chemical contaminants in aquaculture products and feeds: Micro-organism method for the detection of oxolinic acid (OXA) and oxytetracycline (OTC) in fish	EA Tendencia	GOJ-TF
68	Status and needs of primary aquatic animal health care in small scale aquaculture.	Edgar C Amar / (CL Pitogo)	GOJ-TF
69	Investigation of the situation of antibiotics/chemical usage and regulations in aquaculture	RM Coloso	GOJ-TF
Accelerating Awareness and Capacity-Building in Fish Health Management in Southeast Asia			
70	Establishment of novel prophylactic and therapeutic methods for the prevention of viral infections in commercially important maricultured fish.	RV Pakingking, Jr	GOJ-TF
71	Establishment of immunization regimen for the prevention of viral nervous necrosis (VNN) in high value marine broodfish.	RV Pakingking, Jr	GOJ-TF
72	Evaluation of carriers for practical delivery of vaccines to shrimp, *Penaeus monodon* and other crustaceans	EC Amar	GOJ-TF
73	Parasitic and shell diseases of abalone (*Haliotis asinina*) in the Philippines	GE Pagador	GOJ-TF
74	Surveillance and training of fish-borne zoonotic parasite of commercially important freshwater fish in some selected Southeast Asian countries	GE Pagador	GOJ-TF
75	Molecular diagnosis and prevention of economically important viruses in fish and shrimp	LD de la Peña	GOJ-TF
Resource Enhancement of Internationally Threatened and Over-exploited Species in Southeast Asia Through Stock Release			
76	Stock enhancement of Napoleon wrasse *Cheilinus undulatus*	FL Pedroso	GOJ-TF
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AQD's COLLABORATING PARTNERS

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[2]**DOST**, Department of Science & Technology
[3]**UP**, University of the Philippines
[4]**PCAARRD,** Philippine Council for Agriculture, Aquatic, and Natural Resources Research and Development
[5]**UPV**, University of the Philippines Visayas
[6]**JSPS**, Japan Society for the Promotion of Science
[7]**USB**, United Soybean Board
[8]**DOST/NRCP**, National Research Council of the Philippines
[9]**IFS**, International Foundation for Science
[10]**VLIR**, Flemish Interuniversity Council
[11]**The WorldFish Center**, The WorldFish Center
[12]**RIHN**, Research Institute for Humanity and Nature
[13]**GOJ-TF**, Government of Japan - Trust Fund
[14]**RESCOPAR**, Rebuilding Resilience of Coastal Populations and Aquatic Resources
[15]**JIRCAS**, Japan International Research Center for Agricultural Sciences

Producing quality seed for sustainable aquaculture (QS)

International trainees preparing hormones for the induced spawning of grouper (left), injection of the hormones in grouper broodstock (right).

World aquaculture production has increased in the last decade and the ASEAN region is recognized as a major contributor to this growth. Seed production and farming techniques of commercially important aquaculture species have been refined in the region as well as the promotion of the merits of using quality seeds. Good quality aquatic seeds mean fit, free from disease or chemicals, ideally uniformly-sized fry, fingerlings, or juveniles, or for seaweeds, plantlets that subsequently express good performance attributes during culture. These beneficial traits are desirable color, shape, good growth, health, efficient feed conversion, better reproduction, tolerance to and survival in poor or extremely adverse environmental conditions.

The sustainable production of aquatic species for human consumption depends primarily on the availability of good quality seedstock apart from the adoption of optimal husbandry techniques. With the intensification of aquaculture systems and the environmental challenges such as those resulting from climate change, genetic quality and culture management should be considered as equally important in ensuring a steady yield of good quality seeds and later, marketable products from aquaculture. Hence through this program, activities that determine optimal methods in producing sufficient, quality seedstock are being pursued. Researches primarily involve conventional means of stock improvement such as domestication, broodstock management, strain evaluation and selective breeding or genetic improvement of economically important freshwater and marine species. Advanced methods such as molecular marker assessments are likewise being integrated into stock management. These technologies shall be verified and once mature, shall be packaged as viable or cost-effective methods for broodstock and seed production. The ultimate aim is not only to develop and verify technical knowledge but also to train and inform industry stakeholders, especially fish farmers, of protocols for seed production and source of seeds especially of improved stocks. SEAFDEC/AQD's main goal for its QSSA program is to generate, verify and promote technologies to ensure the sustainable production of quality seed stock for aquaculture as well as for stock enhancement. More specifically, the objectives are to (a) develop good quality broodstock for traditional and emerging species through domestication, genetic management and nutritional intervention; (b) improve quality and production of seedstock through the refinement of hatchery and nursery management methods; (c) develop schemes for the production, management, maintenance and dissemination of genetically selected and improved stocks; (d) produce sufficient seedstock through the adoption of economically viable seed production systems; and (e) build the capability of fishfarmers and other industry stakeholders in appropriate breeding and larval rearing technologies through training, extension and information dissemination.

Development of good quality broodstock and implementation of proper stock management protocols

The quality of broodstock is an important factor in seed production for aquaculture and stock enhancement. Stocks must be genetically diverse to ensure fitness when farmed or released in specific environments. For stock release, seeds produced by quality spawners will have to be genetically similar to stocks in the receiving area to maintain the genetic integrity of the population in the enhancement site. Supportive breeding, or use of broodstock from the enhancement site is often suggested to prevent further deterioration of stocks in the release area. Management of genetic stocks in the wild is important as well for these are sources of founders in aquaculture hatcheries. The following are the accomplishments of all the R&D activities that address objective A.

Commercial species

Two consecutive generations of shrimps were on-grown as broodstock for the study on the development of techniques for the sustainable production of good quality captive *P. monodon* breeders. The reproductive performance of both F1 and F2 generation broodstock were not consistent in terms of percentage maturation, successful spawning and fecundity. Three sex ratios (1,2 or 3 males per female) were evaluated to improve hatching rates. No significant differences in the three sex ratios were noted. As regards the ideal stocking density for rearing shrimps to broodstock size, higher survival were obtained for stocks reared at 0.25 ind/m^2 while broodstock size was obtained within 1.5-2.5 months for those stocked at lower densities of 0.5 and 0.25 ind/m^2.

Molecular markers that will identify stocks and consequently aid in determining genetic quality are currently being developed for several commercial aquaculture species. With funds from the Philippine Department of Science and Technology, the University of the Philippines, Tohoku University in Sendai, Japan and SEAFDEC/AQD jointly implement work on the development of quality shrimp, milkfish, abalone and oyster broodstock through stock monitoring and management protocols which incorporate DNA marker information. Individual tissue samples from potential shrimp broodstock sources namely, Zamboanga, Surigao, Palawan, Zambales, Bohol, Himamaylan. Digos, Roxas, Ozamis and Masbate in the Philippines were collected and have been genetically characterized using 7 microsatellite DNA (msDNA) loci. Similar levels of genetic variability were noted in all ten stocks. Molecular marker-based broodstock management method is also being adopted for the Philippine milkfish *Chanos chanos* stocks. Samples (n=50/stock) were obtained from sixteen populations of wild and/or hatchery bred broodstock (including stocks from Indonesia where some seedstock are used in local commercial grow-out operations). Nine novel microsatellite marker loci were developed and identified as utilizable and were subsequently used to delineate stocks. Results of the preliminary screening of genetic diversity in four stocks (from Bohol, BFAR Palawan, Dagupan and

Trainees from Egypt monitoring seabass broodstock maturity by measuring egg diameter in females and the level of milting in males.

Injection of hormone to induce seabass to spawn (left) and washing fertilized seabass eggs with clean seawater before stocking in incubation tanks (right).

SEAFDEC/AQD Iloilo) showed similar levels of expected heterozygosity ranging from 0.678 to 0.703. Twelve remaining stocks are currently being genetically characterized for microsatellite marker variation at the same nine loci. Once complete, data on the genetic differences among these stocks and information on the best possible sources of milkfish broodstock will be known. The impact of domestication on known milkfish hatchery stocks of varying ages and generations such as those being maintained and used at SEAFDEC/AQD for seed production will also be defined. As for the abalone, stocks from Pangasinan, Palawan, Zamboanga del Sur and Masbate have been collected and five families per stock are being produced for genetic assessment. Preliminary genetic evaluation of broodstock from Pangasinan, Palawan and Zamboanga del Sur showed that based on six novel microsatellite markers, genetic diversity as noted from expected heterozygosity, ranged from 0.800 for the Zamboanga del Sur strain to 0.868, the highest being that of the Palawan strain. The highest number of alleles, which is another measure of genetic variability, was observed in the Palawan strain at 12.17 while the lowest was in the Zamboanga del Sur stock (9.67). The higher the genetic variability indices are, the more fit hence the better the stock is. The families produced for each stock will be evaluated for genetic diversity (to include parentage analysis based on msDNA marker variation) and these will later be correlated with production characteristics.

Apart from genetic intervention, nutritional methods to improve egg production and quality have been done through the inclusion of pigments in the diet. A maturation diet for grouper (*E. fuscoguttatus*) has been formulated using a natural pigment such as paprika and feeding trials on its effect on seed quality and production was monitored. Mature oocytes and spermiating males were observed in treatments with pigments (paprika and beta carotene) while no spermiating male was observed in the control. In another run, feeding grouper broodstock beta-carotene or paprika did not improve reproductive performance in the fish. The assumption was that the breeders used were still adapting to the diet and needed more conditioning prior to induced spawning. More trials need to be conducted.

To improve reproduction in the donkey's ear abalone, maturation diets are currently being assessed. The nutrient composition of eggs from wild-sourced abalone and those from hatchery-bred stocks were identified. Dietary formulations with varying levels of protein/energy have been done and their effects on abalone reproductive performance were tested using wild and hatchery conditioned broodstock. Reproductive performance of hatchery-bred abalone broodstock generally improved with an increase in dietary protein energy levels from 27% CP/3210 kcal/kg energy to 37%CP/3350 kcal/kg energy. At the highest level of dietary protein energy tested (42%/3750 kcal/kg energy) however, there was a levelling off of the reproductive performance of the animal. Lower spawning success correlates well with the lower amount of protein and lipid in the egg. A shorter time to maturity was noted with broodstock given formulated diets containing 8 % lipid level sourced from a combination of fish oil and thraustochytrid. Moreover, egg hatchability increased with increased lipid level not withstanding the different lipid sources used. The higher levels of dietary HUFAs and PUFAs correlated well with better reproductive performance.

Efficient low-pollution diets for use in rearing potential freshwater prawn broodstock are being developed and tested. Isonitrogenous (35% crude protein) and isocaloric (346-350 kcal/100 g diet) grow-out diets were prepared. These diets were used for the tank and lake-based grow-out culture of *M. rosenbergii* postlarvae (PL). Broodstock diets were also formulated to be isonitrogenous (38%) and isocaloric (378 kcal/100 g diet). Sardine fishmeal was replaced with cowpea meal at varying levels (Diet 1= 0, Diet 2 = 15, Diet 3 = 30, Diet 4 = 45, and Diet 5 = 60%) in grow-out and broodstock diets (Diet 1 = 0, Diet 2 = 10, Diet 3 = 20, and Diet 4 = 30%). Feeding trials in lake-based cages showed

that specific growth rate (4.52–5.00%/day) and survival rate were not affected by the replacement of cowpea meal protein level in the test diets. Prawns fed commercial prawn diet had survival rate (64%), mean body weight (13.98 g) and specific growth rate (4.73%/day) comparable to the performance of the test diets. Cowpea meal as a replacement for fishmeal has been noted to be acceptable for freshwater prawns when incorporated up to levels of 45%. The economic viability of the use of this alternative protein source shall be determined.

Meanwhile, giant freshwater prawn spawners stocked in tanks at 1 M : 4 F sex ratio and fed treatment broodstock diets (1-4 or 0, 15, 30, or 45% cowpea replacement) showed that after 180 days, the percentage of berried females was highest in broodstock fed Diet 4, followed by those fed Diet 2 and 1, and lowest in broodstock given Diet 3. The average number of larvae produced per hatching event ranged from 4,750 to 6,549 larvae, the highest of which was found in broodstock fed Diet 4 and lowest in those broodstock given Diet 1. Larval survival was comparable among diet treatments.

The sustainable production of marine annelids (*Marphysa mossambica*) was pursued as these serve as potential feed for mud crab and shrimp broodstock. The reproductive biology and life cycle of the marine annelid have been defined. Moreover, various feed combinations of live annelid and annelid meal as feed for mud crab broodstock have been shown to improve reproductive performance. Test diets namely: a) NFP or mussel meat, squid, fish by catch with live polychaete, b) NFNoP or mussel meat, squid, fish bycatch without live polychaete, c) FD10 or formulated diet containing 10% polychaete meal, d) FD20 or formulated diet with 20% polychaete meal; e) NFP+FD10, f) NFP+FD20, g) NFnoP+FD10 and e) NFnoP+FD20 have been prepared and the response of maturing crab females to these dietary treatments are being evaluated.

In the blue swimming crab *Portunus pelagicus*, one of the concerns that need to be addressed is the ideal loading density in transporting non-ovigerous broodstock. This is important particularly in procuring potential broodstock from the wild for use in hatcheries. The results showed that survival rates of *P. pelagicus* did not differ significantly ($P > 0.05$) immediately after transport and 48 h post transport suggesting that it is feasible to transport the crabs at a high loading density (10 pcs/bag) for a 6 h duration.

In the oyster, *Crassostrea iredalei*, protocols for broodstock management and conditioning are being developed to maximize production. Broodstock (400 pcs/treatment) were stocked in four conditioning sites namely 1) T1- estuary in Culajao, Roxas City, 2); T2 - semi-intensive pond in DBS; 3) T3 – tank given 200,000 cells/ml, 3:1 mixed microalgae *Isochrysis galbana* (Ig) and *Tetraselmis tetrathele* (Tt), and; 4) T4 – tank given 100,000 cells/ml, 1:1 ratio of Ig:Tt. The sex ratio of broodstock was 1 M: 3 F. Faster gonadal development was observed in T1 and T2 than in the other treatments. About 30 million eggs and 11million straight-hinged larvae were produced from this first run. The highest number of eggs released was 2.7M eggs/female and hatching rate (HR) ranged from 62-69% when stocked at ≤10 eggs/ml and 35% HR at stocking density of ≥10 eggs/ml.

Species for stock management and enhancement

The Napoleon wrasse *Cheilinus undulatus* is a subject of research at AQD primarily to understand its reproductive biology and consequently enable the production of seedstock for use in stock enhancement activities. Efforts to develop broodstock of Napoleon wrasse have not been successful due to difficulty in stock collection. Meanwhile, activities are being undertaken to study the giant grouper for purposes of broodstock development and genetic stock management. ACIAR is supporting preliminary work on broodstock collection and genetic variability assessment using molecular markers.

Refinement of hatchery and nursery management methods to improve seedstock quality and production

Commercial aquaculture species

Trials were made to determine the appropriate hormone concentrations to induce spawning in the spotted scat. Fish spawned at two successive injections for the treatments of 100ug LHRHa/kg BW and 200ug LHRHa/kg BW at 24 hour intervals but no fertilization occurred.

In the pompano *Trachinotus blochii* (Lacepede), optimum conditions for breeding and seed production were determined. Probiotic addition to live food improved growth and survival of pompano larvae. The enhancement likewise improved larval resistance to stress such as hypoxia and stimulated digestive enzyme activities.

Protocols for the nursery rearing of pompano in cages have been developed. Nursery trials using pompano of different initial size at stocking showed that better growth was observed in fish stocked at <5g when fed a dedicated pompano diet. Comparable growth was noted in bigger size fry fed either pompano feed or a generic high value fish feed. When stocked at 1500 and 2000/cage, comparable growth, survival and duration of culture to reach 50g were noted in the two stocking densities. Another phase of the study will test the suitability of higher stocking densities - 500 (Control), 625, 750, 875 and 1000 fry/m2 or 2000, 2500, 3000, 3500 and 4000 fry/cage, respectively. Trial implementation has been affected by a parasitic outbreak in the stocks which were to be used for the run. Preparations are underway for the next scheduled experiment. Finally, apart from noting the biological outcome of the nursery runs, the costs for production under the various

interventions are being noted to enable determination of the most suitable, cost-effective seed production scheme.

For milkfish, feeding trials involving a comparison in the reproductive traits of broodstock fed fortified vs non-fortified (control) diets were conducted. Fortified diets meant inclusion of phospholipids, carotenoids, vitamin C, beta carotene and arachidonic acid. It was noted that more spawning episodes were observed in young milkfish broodstock (YMB) fed fortified diets compared to the old milkfish broodstocks (OMB; 17-29 year old). However feeding fortified diet to the aging milkfish broodstock did not improve the quantity of eggs produced but the quality of newly hatched larvae were much improved. In contrast, older stocks fed fortified diets produced longer fry but those produced from young breeders showed higher fry survival. Moreover, the fry produced by broodstock fed milkfish fortified diet was resistant to stress specially during harvest, handling and transport. Milkfish fry can stay in plastic basins for a week with minimal mortality.

Questionnaires on fry quality assessment were sent off to clients to determine whether they are satisfied with the seedstock produced by the SEAFDEC/AQD hatchery. Each buyer commented that there was an improvement in the quality of fry they bought, mentioning specifically that fewer abnormalities were observed after the harvest of the fingerlings. The clients also expressed their preference for uniformly sized fry (to exclude size 2 fry) hence additional activities in the hatchery in 2015 will be to sort fry before packing.

To improve the seed yield of selected high value marine fish species such as grouper, red snapper, seabass, rabbitfish and pompano, sodium-iodide enriched rotifers and *Artemia* were used as feed for larvae. Metamorphosis and survival of the same high value species were noted. It was observed that the survival rate of red snapper was improved when fed sodium iodide enriched rotifer (for 16 hours) and *Artemia*. Snapper fed sodium iodide enriched rotifer metamorphose earlier than those fed enriched *Artemia*. The improved survival of red snapper could be due to less stress to the fry during period of metamorphosis. Iodine is necessary for the production of thyroid hormone which plays an important role in the metamorphosis of fish. The fry fed *Artemia* enriched with emulsion +sodium iodide (short and long enrichment) metamorphosed earlier. The present study confirms the experiment on Atlantic cod larvae where rotifers enriched with iodine and selenium given to the fish improved their survival. However, in this study, no clear results were observed in terms of weight gain and increase in total body weight of red snapper fry.

Cocconeis sp, *Nitzchia* sp and *Diploneis* sp have been used as alternative benthic diatom feed species for the hatchery rearing of abalone. Feeding preference experiments involving abalone juveniles were conducted. After 48 hours, the highest percent incidence of juveniles (20.8% juveniles h-1) moved to plates with *Nitzchia* sp, followed by *Cocconeis* sp, *N. ramossisima, Amphora* and finally *Diploneis* sp. but among the five natural food organisms, gut analysis showed that *Amphora sp* was highly digestible (27.49%). It was also noted that significantly higher growth rate was observed in abalones fed *Amphora* sp. Feeding trials were also made to determine the suitable diatoms for 3mm-5mm abalone juveniles. In terms of body weight, significantly higher specific growth rate (SGRBW) was attained by 3-mm juveniles fed *Amphora* sp. (4.7 BW d-1) and *Nitzschia* sp. (4.1 BW d-1) ($p<0.05$) compared to those fed *N. ramossisima* (3.2 BW d-1), *Diploneis* sp. (1.1 BW d-1) and *Cocconeis* sp. (0.97 BW d-1). After 60 days of rearing, highest survival of 71% was recorded in 3mm abalone fed *Nitzschia* sp. No significant difference was observed in growth expressed as SGRSL when 5-mm abalones were fed the various diatom strains ($p>0.05$). However in terms of survival, after 60d of rearing, highest survival of 65% was recorded in 5-mm abalone fed *Nitzchia ramossisima.* The final objective of these studies would be to determine which diatom species would be appropriate in commercial scale hatchery production of abalones.

Another nutritional intervention to improve abalone hatchery production is the administration of microparticulate diets as alternative feed. An agar-bound microparticulate diet has been formulated based on the nutrient profile of post larval abalone. Proximate analysis of the microparticulate diet (MPD) showed higher levels of crude protein at 47.2% and lipid at 8.9% compared with 14.9% crude protein and 2.1% lipid for the diatoms (*Navicula* sp). The use of MPD in 2-ton fiberglass tanks in the abalone nursery showed high % settlement rate (at 37%) with larvae from wild abalone broodstock from Sagay compared with hatchery-bred larvae (25%). Feeding frequency trials in big tanks showed that daily feeding compared with alternate day feeding (every other day or every two days) is needed to sustain abalone survival in the nursery rearing phase. Percentage settlement rate and survival was highest (32% and 62%, respectively) for abalones fed daily.

Meanwhile, another experiment aimed at increasing the survival rate of abalone veliger larvae through the improvement of harvest, stocking density and incubation protocols was done. Three stocking densities (5,000; 15,000; 25,000 larvae L-1) at extended incubation time were tested. After 8h of incubation, highest veliger survival of 97% was attained at 5,000 L-1 compared with 15,000 L-1 (95%) and 25,000 L-1 (94%). Regardless of stocking density, larval survival declined after 16h to 48h of incubation, but higher survival of 43% was attained in 5,000 L-1 stocking density.

In the oyster, different spawning techniques are being compared to determine the optimal method that can provide a steady production of quality oyster seedstock. All methods (dessication, thermal and exposure to UV-irradiated water) induced oysters to spawn but a faster response (15 min) was noted in the thermally manipulated batch (exposure to 34°C) with a higher number of eggs released per mass spawning batch. However fertilization rate was higher in eggs released by dessication method. As for the optimal egg density during incubation, when stocked at densities of

5 and 10 eggs/ml, higher hatching rates of 62 ± 6% and 69 ± 0.20%, respectively were obtained and noted to be higher than when stocked at 15 eggs/ml (38 ± 4.6%) or higher.

When larval rearing performance in tanks subjected to partial flow-through and/or static water was compared, survival was noted to be higher in static water culture. Finally, feeding *Isochrysis galbana* alone or in combination with *Chaetoceros calcitrans* to oyster larvae gave better survival compared with feeding *Chaetoceros calcitrans* alone.

Efforts have been made to further improve mudcrab hatchery schemes. Enhanced larval growth performance was noted in a diet composed of 1% squid meal and 1% annelid meal. The response of mudcrab larvae to this formulated diet will be compared with those fed BP Nippai shrimp formulated diet. As regards the use of antibiotics, experiments on the application of antibiotics, antimicrobial nitrofuran and probiotics in larval rearing were conducted. Larvae treated with antibiotics commonly used in the hatchery still gave the best result since antibiotic treated larvae survived the longest (10 days) compared with the other treatments.

The influence of stocking density and tryptophan-supplemented diets on the survival and growth of mudcrab *Scylla serrata* in the nursery phase is being determined. Based on stocking density, crabs reared for 4 weeks at 30/m² had higher survival (59.7% vs. 47.6%) than those stocked at 50/m². In contrast, crabs fed mussel and basal artificial diet survived better (57.7%) than those fed mussel alone or mussel and artificial diet with 0.5 to 0.75% tryptophan. In another experiment, crabs stocked at 5/m² and 10m² and subjected to the same feeding regimes, survived better in diet 1 or in the mussel and basal artificial diet combination. Survival was better in a lower stocking density of 5/m² than at 10/m². It was noted that tryptophan did little to reduce incidence of cannibalism. Meanwhile, in determining the optimal feeding ratio of natural food (NF) to artificial diet (AD) that can be used during the 1st and 2nd mudcrab nursery phases, higher survival rates (64-69%) were noted in the diet combinations NF:AD of 15:85, 20:80, 25:75 or 30:70. However 100% AD also gave a good survival rate. Survival rates ranging from 80-90% in NF:AD combinations were higher than those fed singly either NF or AD. As regards cannibalism in mudcrabs, strategies for the reduction of cannibalism have been assessed. Net ribbon shelters were observed to be best in controlling cannibalism in mudcrabs than the *Gracilaria*, nets in zigzag and plastic ties. This evaluation, when done at low densities of 30/m² showed higher survival and growth. When claw-trimmed and autotomized crablets were compared with intact crablets, survival was similar between intact crablets and claw-trimmed ones.

Apart from mudcrabs, larval studies have been done on the blue swimming crab (BSC) *Portunus pelagicus*. BSC larvae were fed commercially available shrimp diets. Results showed that there was no significant difference in the survival rate of *P. pelagicus* larvae fed natural food (NF; *Brachionus* and *Artemia*) and various shrimp formulated diets given in combination with natural food (50% NF+50% commercial diet). Likewise, growth indices did not vary among treatments. Antibiotics have also been tested to evaluate its impact in BSC larval production. Survival from zoea 1 to megalopa was highest in those treated with furazolidine (0.2 ppm), every other day (9.58%). Zoea did not reach megalopa without antibiotics. In the confirmatory run, larvae with furazolidine applied daily (9.38%) or every 5 days (7.29%) had higher survival than those larvae with oxytetracycline after every 5 days (5.63%) and daily water change (5.42%). Megalopae were produced (3.96%) even without antibiotics indicating that megalopae can still be produced depending on the quality of the larvae.

For BSC nursery production, initial responses showed that bigger crab instars show high mortality at 8ppt. when laboratory scale tests comparing survival and molt frequencies at 8- 32ppt salinity levels were conducted. To improve the nursery production of the BSC, the optimal stocking density was determined in the two nursery phases. Survival rate of 37% was obtained in the lowest stock density of 300 ind/ton for phase 1. For phase 2, the optimal stocking density in tanks was noted to be 200 individuals / ton. When reared in netcages, survival was best at 54% in 30 individuals/m² for phase 1 and 80% for stocks reared at 10 individuals/m2 for phase 2 rearing.

To enable the promotion of *Kappaphycus* culture, nursery rearing techniques are being refined. Tissue cultured cultivars are produced in the laboratory and reared in the land-based nursery tanks and in sea cages. Optimization of culture conditions for *Kappaphycus alvarezii, K. striatum* and *Eucheuma denticulatum* in-vitro was conducted. Growth rate was determined at different concentrations of $NaNO_3$:10, 5, 1 ppm. There was no significant difference in the growth rates of *Kappaphycus* grown at different concentrations. Lower concentrations were tested (1, 0.1, 0.01, 0.001 ppm) and growth rates of *Kappaphycus* were compared. Growth rates of *Kappaphycus* grown at 1 or 0.1 ppm were significantly higher than those grown in lower concentrations. Meanwhile, the growth rates of explants from different parts of the thallus were tested. Growth of apical tips was significantly higher than the other sections of the thallus. The apical tips are the best part of the thallus as source of explants for micropropagation. Different sizes of explants were also tried: 0.3, 0.5, 1.0, 2.0 cm. Growth of explants with sizes 0.5, 1 and 2 cm were significantly higher than 0.3 cm explants. The best length of explants used for micropropagation is 0.5 cm.

Efforts to outplant tissue culture explants are currently being undertaken. There were 1000 tissue cultured (TC) propagules of *Kappaphycus* transplanted in Panobolon, Cabalagnan, Guimaras. Propagules planted in Bohol, Panobolon and Zamboanga had specific growth rates (SGR) of 7, 6 and 4.3% respectively. Cultivars tested in Bohol, Zamboanga and Guimaras showed heterosity until 3rd pruning. There were propagules planted in Tawi-tawi and and Calatagan, Batangas for test planting; however, TC propagules planted in Calatagan died.

Emerging species for aquaculture

Silver perch *Leiopotherapon plumbeus* is a promising species for commercial inland aquaculture particularly because it is an indigenous species and it commands a good market price. This year, two rotifer strains (*Brachionus rotundiformis* and *B. plicatilis*) were tested as starter food for first feeding larvae. Results suggest that *B. rotundiformis* is a suitable starter food for first-feeding silver therapon larvae. Likewise, a larval feeding protocol for silver therapon is recommended as follows: *B. rotundiformis* from 2 to 13 DAH, *Artemia* nauplii from 14 to 35 DAH, and co- feeding of *Artemia* nauplii and artificial diet from 36 to 95 DAH. Apart from natural food, a microparticulate diet will be evaluated as feed for early stage silver therapon, because in a preliminary trial, early stage silver therapon (8 DAH) were noted to be feeding on microparticulate diet. A microparticulate diet (< 60 µm) will be introduced at 2, 5, 8, 14, 20, or 26 DAH to determine the earliest weaning time of silver therapon larvae onto microparticulate diet employing a co-feeding strategy.

Improvements in the hatchery and nursery production of sandfish *Holothuria scabra* have been undertaken. Refinements in the nursery protocol included modification of the floating hapa nursery design and determination of the optimal stocking density. PVC pipes instead of bamboo-framed modules increased ease of maintenance and monitoring of sandfish in nursery cages. Stocking densities of 30, 60, 90, 125 or 250/m^2 cage showed that survival was highest in the lowest stock density of 30/m^2. Nursery runs are likewise being conducted in three different sites (floating hapas in Igang, Guimaras, in a protected cove in Ajuy, Iloilo and in an open bay in Concepcion, Iloilo). Of the three, the protected cove shows good prospects for nursery rearing.

Species for stock enhancement

The seahorses, *Hippocampus barbouri* and *H. comes* are continuously being propagated in the SEAFDEC/AQD hatchery for possible stock release. Prior to seed production for stock release, genetic analysis of the different seahorse species are being pursued. Tissue samples from seahorses collected from Molocaboc Island in Sagay City and a seahorse hatchery in Japan have been analysed for genetic characterization. Species-specific primers HiSpiF1 and HiBarF1 were confirmed for species identification of *H. spinosissimus* and *H. barbouri*. Meanwhile, another marker system (mtDNA RFLP) was tried and species diagnostic RFLPs were identified mainly for *H. comes*. Apart from mtDNA RFLP, microsatellite markers have been tried for use in genetic stock profiling. Eight polymorphic microsatellite loci from related seahorse species were tested, of which only four loci showed consistent results after preliminary fragment analysis in 4 seahorse individuals. However, when Molocaboc (21-24 individuals) and hatchery-reared (21 individuals) seahorses were screened for population structure analysis, data from only one locus was suitable for FST population comparison analysis. Polymorphic microsatellite loci from other related seahorse species will be tested further.

Development of schemes for the production, management, maintenance and dissemination of genetically selected and improved stocks

Commercial species

Selective breeding programs have commenced for selected crustaceans with support from the Government of Japan Trust Fund (for mudcrab, shrimps and freshwater prawns), SEAFDEC/AQD and the Philippine Department of Science and Technology (for shrimp and the mudcrab). For the mudcrab, stress tests (particularly formalin tolerance and disease challenge response tests) were done to determine strain level differences in the effort of identifying stocks with better fitness attributes. Details on the shrimp and mudcrab studies are not included here but are covered in the GOJ-TF report.

Hybridization is currently being done on the local commercial abalone species (*Haliotis asinina*) by crossing this with other Philippine abalone species, *Haliotis planata* and *Haliotis glabra*, to enable the production of stocks/species with improved traits. After 330 days of culture (DOC), specific growth rate based on body weight were as follows: 0.83% for pure *H asinina*, 0.73% for hybrid *H asinina* x *H planata* and 0.78% for *H asinina* x *H glabra*. Survival was highest for the *H glabra* hybrid followed by the pure *H. asinina* and the *H planata* hybrid. The third hybrid HAFVM (cross of female *H. asinina* and male *H. varia*) is currently being maintained. Meanwhile, broodstock for use in future hybridization activities are being collected, the recent collection (*H. planata* and *H. varia*) was from Palawan. Histological analyses are also being done on the pure strain and the hybrids. Apart from hybrid production, triploidy induction trials using different caffeine concentrations are also being conducted.

Ice-ice disease in seaweeds is a problem. Methods to develop resistant strains of the seaweed *Kappaphycus* and reduce epiphytes are being studied. Trials to expose ice-ice bacteria to haploid and diploid *Kappaphycus* propagules shall continue with the collection of a new batch of bacteria for the assay as preliminary trials showed no induction of infection to the propagules. Another experiment compared growth rate and carrageenan quality of haploids, diploids and tissue cultured explants. Plants from tissue culture had higher growth rate and higher gel strength compared with haploids or diploids. Another activity involves field growth experiments. Growth rates of diploids and haploids were not significantly different when grown in land-based facility. Growth rate of diploids in sea-based facility, i.e. cage, was significantly higher than that of haploids.

Adoption of economically viable systems to produce sufficient seedstock

Several fish/shellfish production projects are being implemented at SEAFDEC to demonstrate the viability of small-scale and/or large-scale seed production systems. Mass production of sex-reversed and mixed-sex Nile/red tilapia fingerlings as well as large scale production of abalone juveniles, are continuously being done. In the small-scale Nile tilapia hatchery, about 1,145,000 Nile and red tilapia fingerlings were produced from 363F and 75M spawners from January 2014 to date. Catfish are also being produced in the same hatchery. This year, a training course on catfish breeding was conducted where nine local participants attended.

For the abalone, juvenile production is on-going. New broodstock have been obtained from the wild (W) apart from the existing hatchery-bred (HB) broodstock. All stocks (299 HB and 172 W) were tagged and currently being monitored. A total of 67,859 abalone juveniles (5mm-8mmSL) have been produced with an average survival rate (from veliger larvae) of 0.68% from a range of 0.065%-2.61%. Abalone juveniles (n=23,075; 1.6cm -3.5cm SL) have been sold contributing PhP 111,980.00 to the Department's production income.

Several experiments (reverse day-night cycle; artificial spawning, use of *Nitzchia* sp as diatom diet and use of microparticulate diet) were planned to further improve abalone juvenile production. The covered tank to be used for the experiment on the reverse day/night cycle is still being constructed. Meanwhile, large-scale production of abalone juveniles was done using *Nitzchia* sp. as diatom feed in 12-ton outdoor tank stocked with 1,500,000 veliger larvae. Using this set-up, the survival rate of abalone juvenile (5mm-8mm SL) was 0.20% yielding 3,033 abalone juveniles.

Capacity-building of fishfarmers and other industry stakeholders on appropriate breeding and larval rearing technologies

AQD scientist Mila de la Pena explaining to on-site trainees of a state college in Zamboanga the culture of live food for mangrove crab

As part of the DOST-supported mudcrab study, on-site technical assistance on mudcrab seed production was provided to several private hatchery operators, namely the Mari-al Hatchery in Quezon, Aquatech Hatchery in Iloilo and the CDO Foodsphere Inc Hatchery in Zambales. Promotion of the hatchery and nursery technologies for mudcrab are also extended to SUCs (State Universities and Colleges) as well as the local government units by way of mudcrab hatchery, nursery and grow-out operations training and on-the-job or internship training courses. A pilot demonstration hatchery was also constructed in Guindulman, Bohol and the technical staff (from the LGU) assigned to start operating this hatchery have already undergone training at SEAFDEC/AQD.

Several specialized training courses were offered to local government representatives, private sector investors and fisherfolk. These were on sandfish, abalone, tilapia and the giant freshwater prawn. Apart from these customized courses, on-the job trainees were also accommodated in the AQD hatcheries during this period. Moreover, technical assistance was also provided to local, international private sector clients through the Agree Build Operate and Transfer Aqua Negosyo Program. The details are to be included in the report of the Technology Verification and Demonstration Division.

Anticipated Impacts

With the bulk of researches focused on broodstock management and improvement, hatchery and nursery refinements utilizing both advance nutritional and genetic innovations, we would expect that information generated from these efforts will definitely be beneficial to the stakeholders. Interventions such as the use of molecular tools in stock management, new strains, novel diets (microparticulate diets, alternative protein sources, low pollution diets) and optimal feeding schemes for every phase of aquaculture production will hopefully translate to improved yields on farm when these technologies are effectively transferred to farmers in the region.

Promoting healthy and wholesome aquaculture (HWA)

Feed developed in SEAFDEC/ AQD (above) and broadcasting formulated feeds in a pond at AQD's Dumangas Brackishwater Station (left)

Healthy and wholesome aquaculture is a thematic program that aims to address the goal of attaining sustainable aquaculture production through provision of protein needs for the growing human population. Although research and development efforts on these aspects have already resulted in phenomenal growth of the sector in the last decades, further studies are needed especially now that we are faced with challenges posed by ecological, economic, and climatic changes. To be assured of more significant improvements and production sustainability for future generation, more relevant work relative to the objectives of the program need to be pursued.

The strategies of the program focus highly on nutrition and feed development that may promote and sustain healthy farmed aquatic animals considering possible prevention and control of diseases, environmental concerns and food safety. Best management and good aquaculture practices giving the least environmental impact should be the basis for an optimized and sustainable aquaculture production.

The goal of the program is primarily to improve and sustain aquaculture production through innovations in nutrition and feed development, management of fish health, and preservation of the environmental integrity of aquaculture sites.

Program objectives are as follows: (a) find alternative protein sources as fish meal replacers; (b) develop effective feeding strategies that will incorporate sound environmental management; (c) develop feed at specific growth stages of fish species for which no artificial feed has been formulated; (d) promote efficient feeding practices and provide adequate nutrition for better appreciation of the concept of feed conversion ratio among fish farmers; (e) investigate the efficacy of probiotics and rationalize the need and application of diagnostics that will ensure biosecurity within culture systems and keep out exotic pathogens, especially trans-boundary pathogens; (f) promote the wider use of conventional diagnostic as well as new methods especially for newly reported, emerging diseases; and (g) find effective alternative safe drugs/chemicals (including natural products) to manage aquaculture diseases in lieu of harmful chemicals and drugs which have been regulated or banned due to quality and safety issues.

Alternative protein sources as fish meal replacers

While fish meal is an ideal nutritional source of dietary protein and lipid in most aquaculture feed formulations for fish species, there is an urgent need to reduce the current total dependence of the feed industry on this expensive and finite commodity of unpredictable supply and cost. Supplies from commercial fisheries are limited and unlikely be able to support the increasing demand for fish meal for a growing aquaculture industry. Thus, to address the first objective of the program, several studies were carried out to try the use of alternative and more

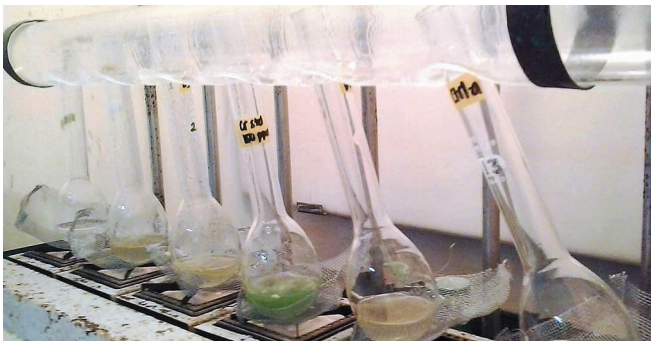
sustainable dietary protein sources in compounded aquatic feeds. Emphasis was focused on the important role played by feed technology and biotechnology in the development and nutritional success of the fish meal replacers put on test, either through the (1) use of fermentation technology to produce single-cell proteins from waste streams with the desired nutrient profile for certain cultured species; (2) use of feeding stimulants to enhance feed palatability and maximize feed intake; (3)use of dietary amino acid to overcome feed formulation imbalances; (4) use of enzyme supplements to aid in digestion, thereby increasing digestibility and nutrient availability; (5) use of appropriate feed processing techniques for the removal/deactivation of anti-nutritional factors.

Fish meal replacers tested include soybean meal, soy protein concentrate, cow pea, mung bean, fermented cowpea (high and low level), fermented ipil-ipil leaf meal (high and low level), in milkfish *Chanos chanos* Forskal diet formulation for the grow-out stage. The use of soybean meal and soybean concentrate as fish meal replacers in the compounded feed given to milkfish in the grow-out stage showed very much improved body weight gain at 2,295% compared to those given the commercial diet which had only 1,682%. Twelve weeks of feeding milkfish in marine floating net cages showed likewise a very much improved growth when cowpea was used as alternate dietary protein source in the diet formulation. Even the use of mung bean showed promising results although growth was not better compared to cowpea. The use of fermented cowpea and unfermented ipil-ipil leaf meal in the formulated diet of juvenile milkfish manifested better growth rate in the animal. Fermentation process in cow-pea based diet appeared to improve the growth performance of juvenile milkfish but not when ipil-ipil meal-based diet was employed. Another alternative protein source that was tried for juvenile milkfish was the distillers dried grains soluble (DDGS). This ingredient was tested and later qualified, however as replacement to soybean meal with as much as 45% replacement in milkfish diet without deleterious effect on growth, feed efficiency and survival of the animal. DDGS as an ingredient has a protein digestibility of 91%, a lipid digestibility of 85%, and dry matter digestibility of 50% which fit the criteria for an excellent feed ingredient.

Milkfish offals, when hydrolysed by using commercial enzyme (*Aroase AP 10B subtilis*) can be an effective alternative protein source in the compounded diet for juvenile grouper *Epinephelus coioides.* It can improve growth and feed efficiency of the animal.

Effective feeding strategies for sound environmental management

Effective feeding strategies for sound environmental management as second program objective was addressed through the following research results using mud crab and giant freshwater prawn as the test animals. Mud crab feed consumption was very much improved when their diets were given in the form of spherical ball and cylindrical shapes. With application of these shapes, the mud crabs were able to facilitate holding and handling of the feed by their claws which resulted in higher feed consumption. More wastage was observed in cube shaped diet because the crab tended to crack the pellet before it could eat it leaving behind small uneaten pieces. Another feeding strategy employed and tested with mud crab was the use of marine-based attractants such as *Acetes*, squid meal, shrimp paste, and spoiled fish flesh both in high and low inclusion levels. Almost all of the diets containing these marine-based attractant elicited positive responses with the animals exhibiting the least time to detect the feed compared with diets that used purified attractants. The use of fish washings to wet the diet during preparation was a significant strategy to improve diet attractability.



Chromic oxide determination in feed and fecal samples (above and right)

Application of acute stressor to grouper fed test diets

Mudcrab holding and eating pelleted feed (arrow).

The use of different grow-out management strategies such as feed management and periphyton-based production to identify production characteristics of the giant freshwater prawn cultured in cages did not give any significant differences in weight, growth rates, and survival when given feed rations at 2, 4, 8, or 15% biomass equivalent. Presence of substrate significantly improved weight and growth rate across all feed rations. The "skip feeding" feed management strategy did not affect the final weight, survival, DGR, and SGR of the animal. However, feed conversion ratio was significantly highest in daily prawn feeding. Increase in substrate area (SA) equivalent to 40, 80, or 120% of cage bottom showed significant effect on prawn yield, with the highest level obtained in SA of 120%. Vertical or horizontal orientation of the substrate did not give any significant effect on production parameters in purely periphyton-based production system. Production parameters were significantly best with prawns stocked at 5 ind/m^2 in a purely periphyton-based production system. Periphyton proximate composition showed highest crude protein content during months of September and October (19 and 20%) and lowest amount of the same nutrient from December to January (12.3 and 13.7%)

Feed development at specific growth stages

Feed development is an important part of the aquaculture industry as it provides the balanced nutrition required by every aquatic species. The development of new species-specific diet formulations supports the aquaculture industry as it expands to satisfy increasing demand for affordable, safe, and high quality finished fishery products. In order to have healthy and wholesome aquaculture products, there is a great need to develop a high quality, nutritionally complete, and balanced formulated diet. To address this particular program objective, feed development for commodities such as golden pompano, *Trachinotus blochii*; mud crab, *Scylla serrata*; abalone, *Haliotis asinina* were initiated. Feed developed for pompano with protein level of 55% and supplemented with crystalline amino acids showed the best growth rate at 2,912% and lowest feed conversion ratio of 1.42.

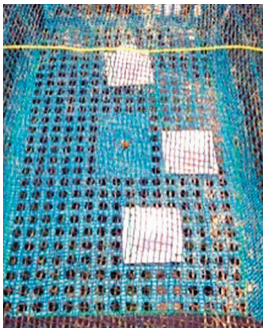
In the case of mud crab feed development, formulated feed containing 48% protein was able to replace 50% or more of its natural food (trash fish). Synthetic binders enabled the mud crab pellet to stay in water for more than 5 hours. Digestibility values showed increased level of crude protein with diets containing synthetic binders despite the use of the same formulation. The binders were shown to contain non-protein nitrogen. Another kind of feed developed was for abalone. They are slow eating animals and require a very stable diet. The need for a more stable diet for abalone, resulted in the refinement of an old formulation with application of a new binder and various feed forms (molo and noodle feed type). Nutrient quality of the 2 feeds were maintained at 30% CP while the water stability greatly differed. The stability of the refined feed

Test of abalone grow-out diets in concrete tanks

Grow-out formulated diets for abalone

Above: Reduction in the incidence of grazing on *G. heteroclada* with addition of *L. calcarifer*

Right and above right: No correlation between water motion and growth of *G.heterocladaas* assessed using clodcards



Higher growth rate in *G. heteroclada* fertilized with 300 ppm NH_4Cl, the pail (left) contains net bags with seaweeds immersed in fertilizer solution

formulation was very much improved using the new type of binder. Average % water stability after 24 hours was recorded at 68% and 47% for the noodle and molo form, respectively, for the refined formulation compared with 38% for both forms in the old formulation. Presentation of both feed either in molo or noodle form, did not affect their efficiency. However, abalone fed the refined feed formulation manifested highest weight gain at 73% and shell length increase at 30% already after only 90 days of culture. Feed conversion ratio was lowest (1.24) for the same batch of feed. The test of feed efficiency in terms of growth, shell length increase, specific growth rate, digestibility is continuing until the 180th day.

Efficient feeding practices and provision of adequate nutrition for better understanding of the concept of feed conversion ratio among fish farmers

Efficiency in every feeding practice is of great essence to best deliver the nutritionally adequate diets to aquatic species to maintain normal growth, health, and reproduction. To address this particular objective of the program, technology verification and production studies for grow-out culture of various commodities in ponds, tanks and cages were carried out. Test fish species included grouper, *Epinephelus fuscoguttatus/ coioides*; rabbitfish, *Siganus guttatus*; soft-shell mud crab, *Scylla serrata*; seaweed, *Gracilaria heteroclada*; white shrimp, *Penaeus indicus*, and Nile tilapia, *Oreochromis niloticus.* Results of the various studies showed that semi-intensive grow-out culture of grouper with SEAFDEC/AQD phased diet feeding in brackishwater ponds is feasible. With an initial ABW of 43.77 ± 15.50g, the stock can grow to ABW of 165 ± 90.0g in 180 days using this type of feeding. The same results were demonstrated in the case of rabbitfish, in a semi-intensive grow-out culture production using formulated diet in brackishwater pond. In 150 DOC rabbitfish with initial ABW of 35g and reared at a density of 0.5m^2 resulted in production of 1265 kg/ha with net income of P 9,243 and ROI of 164% and payback period of 0.53 year in one crop basis. Target production and economic returns were acceptable.

Production protocols for hatchery-reared mud carb juveniles for soft-shell farming were developed. Survival rate of juveniles in net cages ranged from 80-90% for the 5-10 ind/m^2 stocking density. Hatchery-reared *S. serrata*, wild *S. tranquebarica* and *S. olivacea* stocked individually in plastic cages showed an increase in size after molting, both for male and female crabs. Highest weight gain was observed in *S. serrata,* followed by *S. olivacea* and *S. tranquebarica*.

Results on the optimization of the fertilization scheme in pond seaweed culture, showed that *G. heteroclada* enriched with 250 ppm NH_4Cl (26-0-0) had the highest thallus nitrogen content at 2.76% as against the control (unfertilized, UF) which had only 2.13%. Growth trial results had a significant difference between the biomass of UF (1400g) and F seaweed (1633g) at 28 DOC. To test the use of a bio-control, *Lates calcarifer*, in the intertidal culture of *G. heteroclada*, no incidence of grazing such as cut thallus, was observed at 30 DOC. However, growth of *G. heteroclada* and seabass were both poor which may suggest that there could be other factors that limit the growth of seaweeds aside from grazing. The possible effect of water motion on the growth of *G. heteroclada* was monitored at 21 DOC, but data showed that growth and water movement were not significantly correlated.

There is no difference in fecundity and hatching rates of spawns from wild and

hatchery-bred *Penaeus indicus* broodstock. However, high correlation existed between the body weight and the number of eggs produced. No significant difference was found in the ABW of the shrimp cultured for 60 and 90 days. Shrimp cultured for 60 days yielded higher survival at 82% and lower FCR at 1.8 compared to shrimp cultured for 90 days with survival of 78% and FCR of 3.4. There was about 10% occurrence of Abdominal Segment Deformity Diseases (ASDD) in both tanks and pond-reared animals. Economic analysis for 60 days shrimp culture was more viable than a 90-day culture period. The shrimp produced after 60 days were small, but the survival was high. The culture strategies can still be refined.

PL_{18} hatchery-bred *Penaeus indicus* in grow-out ponds at DBS, SEAFDEC/AQD

Monitoring of body weight and total length of *P. indicus* every 15 days

Harvest of *P. indicus* at 60 and 90 days of culture

Anticipated impacts of feed development

Studies under this program have shown a holistic approach in the improvement of aquaculture production through the efficient use of feed ingredients and aquatic feeds that follow strict regulations in their manufacture. Identification of suitable alternative protein sources that may reduce the dependence of feed formulations on fish meal and other fish-based products be attained, thus making feed production in the region more sustainable. Further, research findings under this program may aid in the development of effective feeding strategies and sound environmental management. Development of cost effective aquaculture feed for selected fish species at specific growth stages especially for species wherein no artificial formulation is available, may result in more aquaculture production, satisfy increasing demand for affordable, safe and high quality fishery products and boost the income of small scale farmers and other stakeholders. Lastly, promotion of efficient feeding practices and provision of adequate nutrition for better appreciation among fish farmers of the concept of feed conversion ratio in pond production may improve fish farm productivity resulting in healthy and wholesome produce.

Efficacy of probiotics

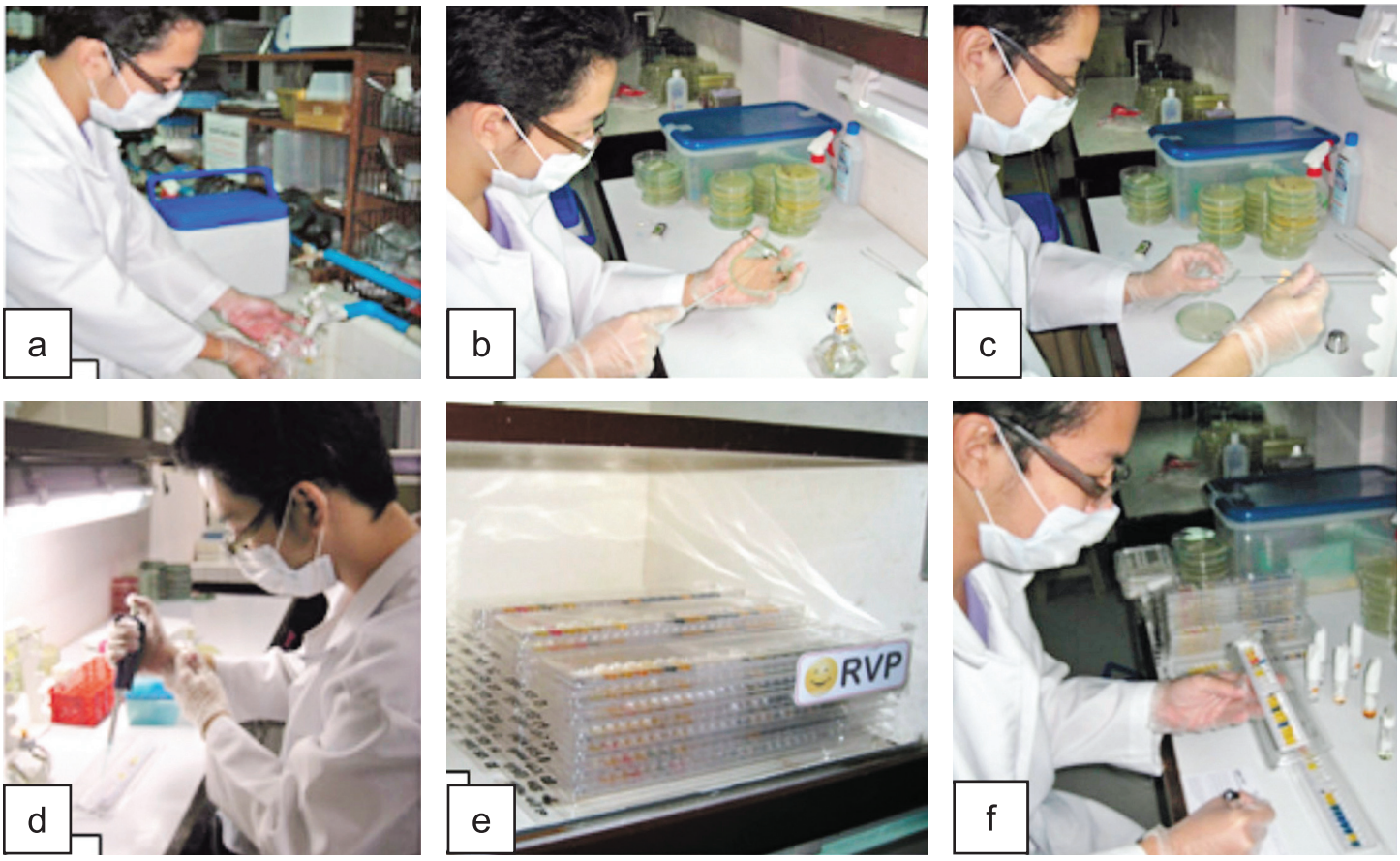
To determine the effects of different levels of intracellular polyhydroxybutyric acid (PHB) on the probiotic capacity of *Bacillus* sp. JL47, suitable growth conditions that allowed the bacteria to accumulate different levels of intracellular

Mudcrab samples showing discoloration of the abdominal region

PHB were established. *Bacillus* sp. JL47 was grown in Luria Bertani (LB) medium and in LB added with 2% glucose. These conditions resulted in two different C/N ratios that affected the PHB accumulation in *Bacillus* sp. JL47. PHB accumulation was significantly higher at 48 h (54.7%) and 72 h (47.3%) of culture in LB + 2% glucose. *Bacillus* sp. JL47 containing different levels of intracellular PHB were administered to gnotobiotic *Artemia* and subsequently challenged with 10^6 CFU ml^{-1} of *Vibrio campbellii* LMG 21363. *Bacillus* sp. JL47 significantly improved the growth and survival of gnotobiotic *Artemia* compared with the control. Survival of *Artemia* was higher in *Bacillus* sp. JL47 containing higher PHB accumulation (54.7% PHB) compared with *Artemia* receiving the *Bacillus* sp. JL47 with lower PHB accumulation (34.6% & 43.7%). Additionally, the PHB-producing *Bacillus* species (JL47) was taxonomically identified as *Bacillus cereus* using 16s rRNA gene sequencing.

Conventional and new diagnostic methods for newly reported, emerging diseases

A database of aquatic pathogens occurring in the country was recently instituted to ensure sustainability of the Philippine aquaculture industry. Prototype database software was developed using 2013-2014 Fish Health Section's diagnostic data. Pertinent literatures concerning each pathogen were compiled from available sources including diagnostic manuals, journal publications, diagnostic reports, OIE disease list, NACA disease list, and disease cards and uploaded in the database. In addition, to establish epidemiological information including genetic, phenotypic, ecological, spatial, and temporal characteristics of aquatic pathogens, shrimp samples were collected from Negros Occidental, Cebu, and Bohol. The distribution of shrimp farms in the 13 regions of the Philippines was also mapped. A total of 447 bacterial isolates, recovered from apparently healthy and diseased shrimps, were identified using conventional (biochemical) and molecular (sequencing) methods. Shrimp samples were also collected and tested for WSSV and other viral pathogens by PCR. Frozen aliquots of positive samples were stored at -80°C for later processing. Reinfection experiments via oral inoculation and intramuscular injection were also conducted to obtain purified virions for extraction of genomic DNA for sequencing. Purified virus bands from PCR positive samples were obtained by ultracentrifugation of the homogenates layered on sucrose gradient. Shrimps experimentally infected with WSSV were further analyzed by transmission electron microscopy to characterize and



Processing of pure bacterial isolates for biochemical characterization test using API 20 E/ API 20NE. Isolates stocked at -80°C are quickly thawed (a) and then streaked on TSA plates (b). After 24 hours of incubation at 28°C, the bacterial colonies are further subcultured on TSA plates (c). Twenty-four hour old bacterial colonies are suspended in normal saline solution and inoculated into the API20E test kit (d). The plates are then incubated at 28°C (e) and test results are read after 24-48 h of incubation (f)





Sampling stations in Brgy. Cagay, Roxas City where oyster samples are randomly collected every month for bacteriological examinations

visualize the viral particles. Furthermore, to lower the cost and improve the efficiency of shrimp pathogen detection via a 'point-of-care' diagnostic, a study on the development of diagnostic tools using nested PCR and lateral flow strip biosensors (LFSB) coupled with a mobile app and cloud-based information management is currently being undertaken.

Diseases affecting farmed and wild mud crab in the different provinces of the Philippines, namely, Albay, Bataan, Cagayan, Camarines Norte, Camarines Sur, Capiz, Catanduanes, Lanao del Norte, Misamis Occidental, Northern Samar, Pampanga, Pangasinan, Quezon, Sorsogon, Surigao del Sur, Zamboanga del Sur and Zamboanga Sibugay were also surveyed using conventional and molecular methods. A total of 767 mudcrab samples were collected from different environments (poly/ monocultured pond, aquasilviculture and natural bodies of water) and processed for bacterial and fungal isolation, parasite and viral detection. External examinations showed several abnormalities such as short abdominal flap, discoloration (blackish, rusty, reddish/pinkish) of the abdominal region, darker carapace, necrotized holes on carapace, claw and legs; presence of fouling organisms, and presence of barnacles *Balanus* sp. on the carapace. Internally, necrotic gills, black/brown gills, and discolored gonads (black, greenish) were noted. Also, stalked barnacles, *Octolasmis* spp. were observed in the gills. The intensity (number of parasites in a single infected host) was highest in mudcrab collected from aquasilviculture sites, followed by those from the wild and pond. Prevalence was highest in mudcrabs from the wild (23%) followed by those collected from ponds (16%) and aquasilviculture sites (16%). A total of 1450 bacteria were isolated from the hemolymph of the sampled mudcrabs. Identification of the bacterial isolates using conventional methods is on going. No fungal infection was observed. Mudcrab samples were also submitted for viral detection using molecular techniques; out of the 200 samples examined, 11 were positive for WSSV while the rest of the samples examined were negative for infectious hypodermal and hematopoietic necrosis virus (IHHNV), taura syndrome virus (TSV), yellowhead virus/ gill associated virus (YHV/GAV), and infectious myonecrosis virus (IMNV).

The quantity and composition of the bacterial microbiota in the rearing water, sediment, gills and intestines of tilapia *Oreochromis niloticus* collected every 2weeks from Day 15 to Day 135 after stocking for grow-out culture in 6 earthen brackish water ponds in the Philippines were also investigated. The total heterotrophic aerobic bacterial counts obtained in the water, sediment, gills and intestines of tilapia ranged from 10^3 –10^4 c.f.u. ml^{-1}, 10^3 –10^5 c.f.u. g^{-1}, 10^5 – 10^7c.f.u. g^{-1} and 10^4 –10^7c.f.u. g^{-1}, respectively. The dominant bacteria (n=545) isolated from the water, sediment, and as well as gills and intestines of tilapia, were characterized biochemically using the conventional methods and commercial kits (API 20E/ API 20NE). In terms of composition, a total of 20 bacterial genera and 31 species were identified with the preponderance of gram-negative bacteria constituting 84% of all bacterial isolates examined. The composition of intestinal and gill bacteria of tilapia examined were apparently similar; predominantly *Aeromonas hydrophila*, *Bacillus* spp., *Plesiomonas shigelloides*, *Shewanella putrefaciens*, *Pseudomonas fluorescens, Vibrio cholerae, Staphylococcus* spp., *Pasteurella pneumotropica*, *P.putida*, *Enterobacter cloacae* and *V. vulnificus*. In the pond water, the dominant bacteria

identified belong to *A. hydrophila*, *Bacillus* spp., *V. cholerae*, *Plesiomonas shigelloides,E. cloacae, V. fluvialis, S. putrefaciens, Staphylococcus* spp., *V. vulnificus,* and *A. sobria*. On the contrary, *Bacillus* spp., *A. hydrophila, P. fluorescens, Staphylococcus* spp., *A. salmonicida, A. sobria, P. pneumotropica, P. shigelloides,* and *Stenotrophomonas maltophilia* were the dominant species identified in the pond sediment, indicating that bacterial composition in the pond water and sediment accordingly reflects the bacterial composition in the gills and intestine of tilapia.

To generate baseline information on the sanitary quality of oysters and their culture environments in Panay, 3 major oyster-producing areas comprised of 12 sampling stations, i.e. Brgy. Culajao (3 Stations) and Brgy. Cagay(5), both located in Roxas City, and Brgy. Cabugao (4), located in the municipality of Ivisan, were examined monthly over a period of 9 months, i.e. May to December 2014. Results revealed higher coliform counts in the water samples collected from all stations in Brgy. Culajao and Brgy. Cagay during the warmest month (May) with counts ranging from 33 to 920 MPN/100 ml. In contrast, coliform counts in Brgy. Cabugao in May were within the acceptable range (<2 to 13 MPN/ml) set by the United States Shellfish Sanitation Program Shellfish Harvesting Area Classification Criteria (Approved areas/ No treatment required: ≤43 MPN/100 ml). Also, coliform counts obtained for all sampling stations from June to December ranged from <2 to 79 MPN/100 ml. Similarly, *E. coli* counts in oysters' meat and intravalvular fluid were higher during the warmest months, i.e. May and June, with counts ranging from 210 to >24,000 MPN/100 g. The range of *E. coli* counts quantified in oysters' meat and intravalvular fluid during the rainy months (August to October) were also similar to those obtained in May and June. *V. cholera* was not detected in any of the samples examined whereas *V. parahaemolyticus* counts were within the acceptable range. Current data clearly indicate that these culture areas in Panay accordingly fall under "Category C" according to EU Shellfish Harvesting Area Classification Criteria. This means that oysters harvested from these areas are still safe for human consumption provided that they undergo prolonged relaying and depuration procedures. Importantly, quantities of heavy metals (Hg, Cd, Zn, Cu, Pb, and Cr) in oysters' meat during the wet season were within acceptable limits. This finding is important considering that heavy metals could not be removed from oysters' flesh by depuration. It was also noted that quantities of organic matter and sulfur in the sediments of the sampling sites examined were apparently above the acceptable limits. This is not surprising because stake method has been predominantly practiced in these areas.

Effective and alternative safe drugs and chemicals to manage diseases

To reduce disease incidence in mudcrab hatchery and grow-out, crude extracts from leaves of terrestrial plants, i.e. *Nephelium lappaceum (rambutan)*, and *Terminalia catappa (talisay)* obtained locally from different areas in Panay, were tested *in vitro* for their antibacterial activity against *Vibrio harveyi* and *Escherichia coli* using the agar disc diffusion method. Results showed that both *N. lappaceum* and *T. catappa* exhibited potent antibacterial activity. Crude extracts obtained from these 2 plants were further tested for their toxicity to determine the proper dose for *in vivo* administration in different larval stages of crab. By immersion, zoea and megalopa were very sensitive (100% mortality) to extracts of *T. catappa* and *N. lappaceum* at all test concentrations (500-4000 mg/L). For juveniles (1.78 cm CL), both extracts were non-toxic at 1000 mg/L. When injected in juveniles (0.99 cm CL), *T. catappa* extracts were non-toxic at 1000 mg/L, whereas it is non-toxic up to 20 g/L when given orally.

Bacterial isolates obtained in 2013 were also tested for their pathogenicity to screen for potential probionts. Pathogenicity tests were conducted for different stages and sizes of the mangrove crab *Scylla serrata*. The stages and sizes for the test included zoea, megalopa, and crablets with 0.44, 0.98, and 2.67 cm carapace length. Four different genera of bacteria (*Staphylococcus* sp., *Corynebacterium* sp., *Aeromonas* sp., *and Vibrio* sp.) were administered to determine their median lethal doses in different larval and juvenile stages of the mangrove crab. The bacteria were all pathogenic to zoea with LD50s ranging from $10^{3.4}$-$10^{4.7}$ cfu/ml in a 1-h bath challenge. The megalopae were even more sensitive to the test bacteria with LD90s ranging from $10^{3.1}$-$10^{4.1}$ cfu/ml. Smaller crablets (0.44 cm CL) were more susceptible to *V. harveyi* (LD50=$10^{6.3}$ cfu/ml) than bigger crablets (0.98-2.67 cm CL) (LD50=$10^{7.4}$-$10^{7.9}$ cfu/ml) in a 1-h bath challenge. Additional isolates were obtained from crab intestines using media and methods that are selective for *Lactobacillus* spp. and *Bacillus* spp. Pathogenicity tests were conducted on additional isolates from Pampanga and Lanao del Norte. There were at least five *Vibrio* spp. that were avirulent and would be suitable as probionts after further characterization. More than 10 *Bacillus* spp. were isolated from crabs collected from Roxas City. Pathogenicity of *Bacillus* spp isolates was determined in crab larval stages and in juveniles by bath-immersion and injection challenge. Putative probionts as determined above were tested for *in vitro* antibacterial activity (stab-on-agar-test, SOAT) and quorum sensing inhibitory (QSI) activity. *Bacillus subtilis* strains G100R11, H105, and H10R1 had high QSI activity (15-16 mm) comparable to azithromycin (18 mm). Highest inhibitory activity was exhibited by *Bacillus subtilis* strain G0R11 (10 mm) and lowest by *Bacillus* sp strain G0R3 (6 mm) compared to azithromycin (14 mm), when tested by SOAT. The rest displayed no inhibition zones. The efficacy of G100R11 in preventing/alleviating bacterial infection in crab instar was subsequently determined. Results showed that G100R11 was able to protect crab instar from *Vibrio harveyi* challenge (1 LD50) when added at 10^{7}-10^{8}cfu/ml. However, mortality was recorded when it was added at 10^{3}-10^{6}cfu/ml.

Maintaining environmental integrity through responsible aquaculture (MEITRA)

This thematic program addresses issues on the negative impacts of aquaculture on the environment and how these impacts will be minimized. The goal of this program is to develop environment-based aquaculture technologies by integrating environmental factors in SEAFDEC/AQD research activities and to maintain environmental integrity by promoting responsible aquaculture practices. The objectives of MEITRA are to: (a) assess impacts of aquaculture on biodiversity, and water and sediment qualities in the culture areas and adjacent ecosystems both in marine and freshwater systems; (b) identify appropriate extractive species that may be used in **Integrated Multi-Trophic Aquaculture** (IMTA); (c) develop and promote efficient and suitable environment-friendly culture systems; and (d) conduct biological and ecological studies on species with potentials for resource enhancement.

Assess impacts on biodiversity, water and sediment qualities in culture areas and adjacent ecosystems

As a first step to accomplish this objective, the different stations of SEAFDEC/AQD is undergoing regular monitoring with the following accomplishments.

Igang Marine Station (IMS) and its surrounding area: The bathymetric profile of various stations incorporating seagrass and coral cover information has been completed. Sediment size analyses have also been completed. Above-the substrate water samples will be collected for nutrient analysis in all 36 stations. The biodiversity of the various ecosystem types as well as near the cage set-up has been determined with 805 species in 292 families in 20 major taxa identified in 2013. Benthic fauna inventory revealed a total of 151 individuals of 51 unique taxa represented into six benthic groups (marine snails, bivalves, shrimps & crabs, polychaetes, amphipods, brittle stars, tubeworms) have been identified

Tigbauan Main Station (TMS): Sampling of the shore areas around TMS is being conducted. Some 543 species in 203 families in 10 major taxa have been collected or photographed. Work was started on a book on the marine biodiversity in Tigbauan, Iloilo, illustrated mostly with photographs of species harvested by nearshore fisheries.

A study was conducted to determine the impact of aquaculture activities on the biodiversity of fish and plankton in the area around Binangonan Freshwater Station (BFS). Dominant phytoplankton in the first half of October 2014 were the cyanophyte *Microcystis* and *Oscillatoria* and in the later half, the centric diatoms *Coscinodiscus* and *Cyclotella* in both the East Cove (EC) and West Cove (WC). Nauplii of copepods dominated both sampling periods in WC. In addition to copepod nauplii, *Polyarthr*a was a co-dominant in the first part of October and *Centropyxis* in the latter half, also in WC. In EC, *Diaphanosoma* and *Trichocerca* were the dominant zooplankton in the first and second half of October, respectively, followed in both periods by the nauplii of copepods. Phytoplankton density were observed between the WC (4.0 x10^6 counts/L) and the EC (2.9x10^6 counts/L). Zooplankton counts showed no significant differences between the two sites, although lower in WC (2785 counts/L) than EC (5238 counts/L) Except for species richness, *S* (EC=19.5; WC=18.8), mean diversity indices for phytoplankton such Shannon-Weaver Index, *H'*, (EC=1.42; WC=1.24) and Evenness, *J'*, (EC=0.48; WC=0.42) and Simpson's Index, *l*, (EC=0.36; WC=0.45) were significantly different between the two sites. For zooplankton, no significant differences were observed between the two sites for all diversity indices (*H'*, *J'*, *S*, *l*).

Samples from the fish traps set at the EC and WC collected twice a month from April 2013 to October 2014 showed that the catch from EC ranged from 422 to 117739 g/d (mean of 20698 g/d) was significantly higher than WC which ranged from just 40 to 5642 g/d (mean of 719 g/d). This could be due to the fishermen operating in the open WC daily which resulted in lower biomass caught in the fish trap; whereas the EC is protected from access by open water fishermen. A sharp increase in fish biomass caught in both sites was observed, but especially in EC, since Typhoon Glenda in mid-July. Based on biomass, significantly higher *H'* (EC=0.52, WC=1.09), *J'* (EC=0.28, WC= 0.54) and s (EC=6.6, WC=8.5) was observed in WC than EC. Significantly lower *l* was observed in WC (0.45) than EC (0.74) indicating higher diversity in WC. In EC specifically, the dominance of introduced cultured species is very evident compared with WC. In WC, invasive alien species (non-aquaculture) tended to be higher in dominance than introduced cultured species. Nevertheless, higher dominance of native species in WC was noted compared with EC (Fig.1).

A project on the biodiversity in the milkfish and shrimp fry (semilya) fishery in the surf zones in southern and western Panay is also being implemented. Examination of 24 fry samples from 24 one-hour operation of fry sweepers from 6 fry collection sites showed the following ranges of catch per sample: 8-36 species of fishes and crustaceans, including 47-815 milkfish *Chanos chanos*, 1-537 tarpon *Megalops cyprinoides*, and 1-149 tenpounder *Elops hawaiiensis*. Also captured were slipmouths *Leiognathus* spp., mullets *Valamugil* spp., silver perch *Therapon jarbua*, spotted scat *Scatophagus argus*, snappers *Lutjanus argentimaculatus* and *L. johnii,* rabbitfishes *Siganus fuscescens* and *S. spinus*, and penaeid and palaemonid postlarvae.

Identify appropriate extractive species for IMTA

Studies on various extractive species for use in IMTA such as the sandfish *Holothuria scabra*, the bivalve mollusk *Anodontia phillipiana* and the seaweed *Gracillaria bailinae* have been completed in 2013.

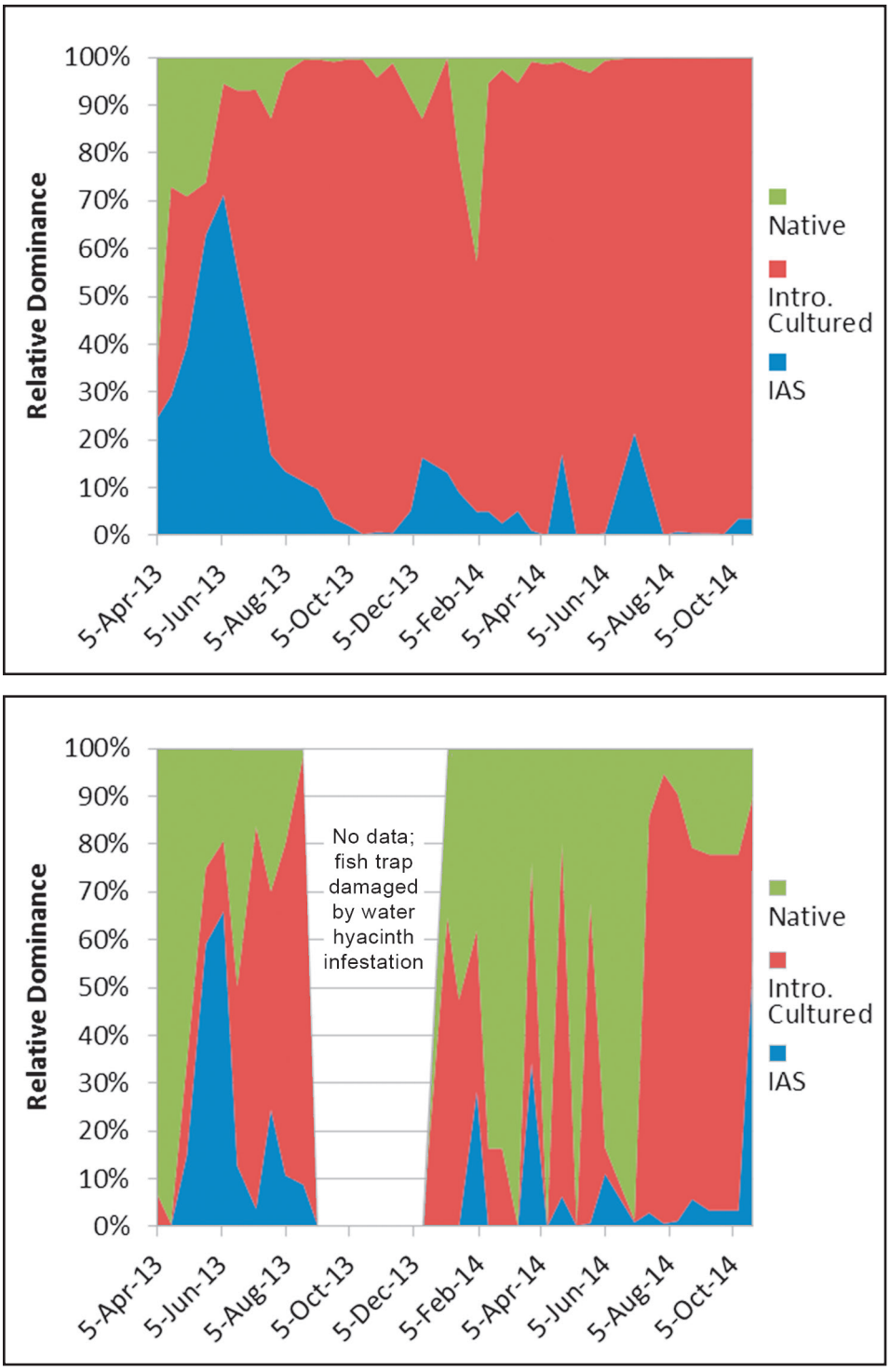


Fig. 1. Relative dominance of native, introduced cultured and invasive alien species in East Cove (top) and West Cove (bottom) of Laguna de Bay, Philippines.

Develop and promote efficient and suitable environment-friendly culture systems

Studies on the culture of the sandfish *Holothuria scabra* is being continued this year. In addition, abalone *Haliotis asinina* grow-out studies particularly in small islands and communities recently started. Refinement of grow-out techniques for the slipper oyster under Philippine conditions was also started.

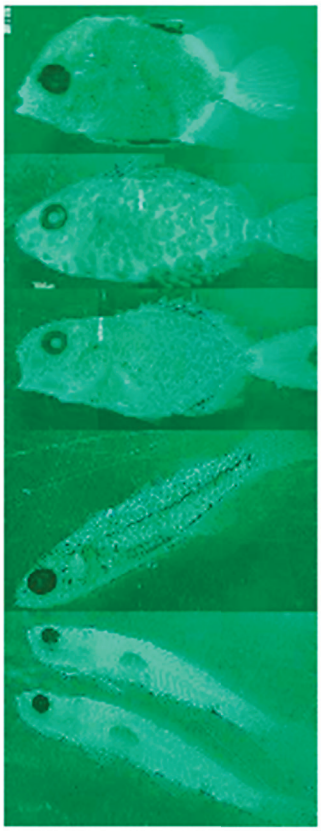
Experiments were conducted to determine optimal conditions for the culture of the sandfish *Holothuria scabra*. In the previous years, substrate type, salinity, and stocking density experiments were done to assess growth and survival of sandfish juveniles. At 30 DOC, sandfish juveniles showed higher growth and survival rates in sandy sediment of 0.89g•d-1 and 98%, respectively, than in muddy sediment with growth of 0.73g•d-1 and 92% survival in the selected experimental sites. Another experiment was started in May 2014 on stocking density using 12, 25, and 50 ind•m-2 using juveniles (M=1.06 g). Initial results showed that growth is stocking density dependent with the best growth observed in the lowest stocking density and the poorest growth in the highest stocking density. Survival was not affected by the aforementioned stocking density which ranged from 88 to 91.5%. Longer-term culture was attempted for sandfish in ponds using a 3x9 pen with re-conditioned sand substrate. A total of 193 individuals (24g mean wt) were used for culture, aiming to produce a single-batch of adults for broodstock experiments. Good growth was observed in the first month of culture from May to June but slow and negative growth was recorded up to the third and fourth month in August and September. With good weather and regular water management in the ponds, survival was high at 96% after the first month and 92% after the 2nd. However, intermittent and sometimes prolonged rains in August and especially in September eventually caused total mortality of stocks. Even with regular tidal water management, mortality was still inevitable due primarily to stratification of the water column. For pond culture of

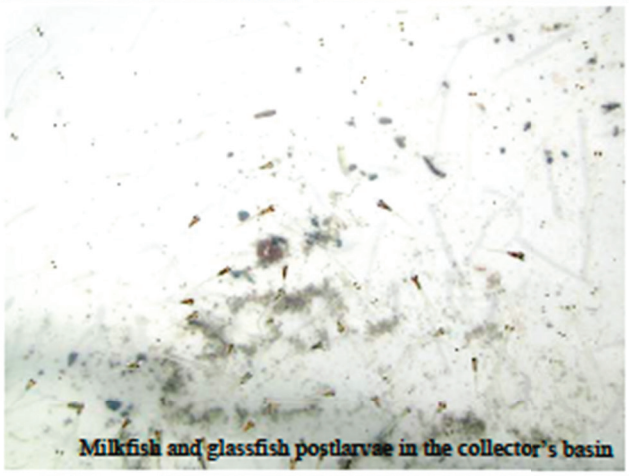
Biodiversity in the milkfish fry fishery in Panay, Philippines

The traditional milkfish fry fishery still supplies seed to brackishwater aquaculture ponds in the Philippines. Although crucial to aquaculture, the fry fishery wastes large numbers of larvae and juveniles of fishes and crustaceans, many of which are commercial fishery species at older and larger sizes, or are food items for nearshore fauna. About 80 species were found in 38 samples from 22 fry sweepers operated at six fry grounds in Southern and Western Panay; some are shown here.

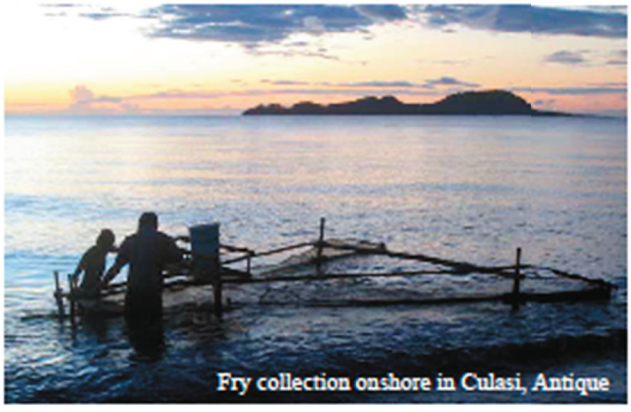
Milkfish, tarpon, and tenpounder postlarvae

Fry collection at a river mouth in Hamtik, Antique





Milkfish and glassfish postlarvae in the collector's basin



Fry collection onshore in Culasi, Antique

sandfish in the Philippines, it seems that the use of some aeration/water mixing system is needed. This will be considered in future plans.

Re-assessment of sea ranching site in Polopina, Concepcion, Iloilo was done to compare the status of the habitat in terms of suitability for sandfish ranching, substrate quality and biota composition after it was hit by typhoon Haiyan. Sea grass cover was still high and dominated by *Enhalusa coroides* but blades were mostly cut after the typhoon. Sediments were also coarser compared with previous assessments of sand-muddy-silty type. No wild sea cucumbers were found in the area a month after Haiyan but another assessment 3 months later showed some recovery where four species of sea cucumbers were found at an estimated combined density of 4 individuals ha^{-1}.

Recently, zoning and delineation of the sea ranching site was done and a draft management plan was formulated during the meeting with project partners. Site markers and monitoring hut/watch tower for the sea ranching site were constructed in collaboration with the Sitio Lo-oc Small Fisherfolk Association (SILOSFA) and LGU of Concepcion.

An Executive Order signed by the municipal mayor of Concepcion was enacted in June, designating the 5-ha pilot sea cucumber sea ranching site at Polopina Island. Within this Executive Order was the creation of the Technical Working Group to oversee the general management of the area.

An annual project evaluation meeting for the ACIAR sandfish project was held in May. Project results and new protocols were discussed. As a project standard, a fixed 49-point environmental monitoring grid was laid out to cover the whole sea ranch site which will be used for physico-biological monitoring to be compared across project sites. Baseline data from this new grid were already taken in June.

Table 1. Nursery culture trials for abalone in different containers.

Culture Container	Shell Length, cm	Body weight, g	Survival
Reef flat (DOC=90d)			
Mesh cages	2.88 ± 0.35	5.02 ± 1.80	42.93 ± 0.13
Trays	3.14 ± 0.37	6.34 ± 1.78	98.64 ± 0.56
Oil containers	3.15 ± 0.43	7.35 ± 3.40	72.56 ± 12.12
PVC tubes	3.21 ± 0.35	6.6 ± 2.21	85.43 ± 2.69
Tanks (DOC=75d)			
Mesh cages	3.03 ± 0.37	6.50 ± 2.34	98.96 ± 0.73
Trays	3.09 ± 0.45	6.46 ± 2.58	98.64 ± 2.03
Oil containers	3.27 ± 0.45	7.83 ± 2.93	98.60 ± 1.27
PVC tubes	3.20 ± 0.43	6.87 ± 2.75	98.29 ± 0.64

Table 2. Grow-out trials for abalone in different containers.

Culture Container	Shell Length, cm	Body weight, g	Survival, %
Reef flat (DOC=120d)			
Mesh cages	4.19 ± 0.03	18.40 ± 0.42	94.72 ± 1.71
Trays	5.16 ± 0.05	31.52 ± 0.88	99.09 ± 0.91
Oil containers	4.89 ± 0.07	27.60 ± 1.06	86.36 ± 12.50
PVC tubes	4.89 ± 0.05	26.76 ± 0.77	99.43 ± 0.57
Tanks (DOC=135d)			
Mesh cages	4.53 ± 0.02	22.35 ± 0.39	99.04 ± 0.16
Trays	4.70 ± 0.05	25.12 ± 0.93	100
Oil containers	4.77 ± 0.05	25.26 ± 0.91	99.09 ± 0.91
PVC tubes	4.77 ± 0.04	25.12 ± 0.68	99.43 ± 0.57

A watch tower and monitoring station was also built within the sea ranch area.

A trial nursery run was started to evaluate adoptability of IMS nursery design in Concepcion. Three nursery hapas were deployed and evaluated. Initial monitoring showed fair growth. However, waves coupled with some debris (bamboo branches, *Sargassum*, garbage) caused problems in the integrity of the nursery structures. Also, high proliferation of barnacles on hard surfaces caused some tearing on the fragile hapa nets. Counter measures have been implemented like installation of protective outer net skirts. The net skirts were effective in protecting the nursery hapas and keeping unwanted debris from entering the nets.

A total of 28,000 1st stage sandfish juveniles (0.01g) were stocked in floating nursery hapas at both Concepcion and Ajuy nursery sites in August. The rearing encountered various problems like intense fouling, predation, net damages, rain and waves that caused mortality and/or escapes. After 30 days, only 8823 individuals were accounted for with weights varying from 0.2-7 g. Of these, a total of 5978 juvenile sandfish (2 g ave wt) were stocked in secondary nursery pens at Concepcion sea ranch site to thin out the hapas. This was the first attempt to conduct secondary nursery rearing in pens on site.

For the abalone experiments in both nursery and grow-out, different types of containers (perforated plastic trays, empty oil containers, polyvinyl chloride tubes and plastic mesh cages) were tested simultaneously in reef flats in Agho Island, Concepcion, Iloilo and in concrete tanks at the Tigbauan Main Station of SEAFDEC/AQD. Results of a 90-day trial in reef flats and 75 day trial in tanks showed that the most suitable container for use in the density experiments to be conducted were PVC tubes for reef flats and oil containers for tanks. Used oil container is recommended for use in tanks because of the highest mean shell length and body weight attained by the abalone after 75 days of nursery culture. In contrast, PVC tubes were chosen for the reef flat because of the highest mean shell length attained after 90 days of nursery culture. Although second highest only to container and tray

in terms of body weight and survival, respectively, it is the most appropriate for use in the field because it is sturdy and can withstand wave action compared with the other 3 containers. Table 1 summarizes the results of the nursery container trials while Table 2 summarizes the shell length, body weight, and survival of abalone in different containers during the grow-out trials.

Preliminary results of grow-out experiments in reef flats (120 d) and in tanks (135 d) show that abalone reared in mesh cages showed poor growth performance in terms of SL and BW compared with those in other culture containers. Survival rates however, remained high irrespective of the container used. This grow-out culture is still on-going.

For the stocking density experiments for nursery in reef flats (PVC tubes) and tanks (oil containers), abalone were stocked at 100, 200, 400, 800 and 1000 indiv. m^{-2}. Preliminary results of the density culture trials for the nursery phase in both tanks and reef flats showed that SL and BW were inversely proportional to density, while survival remained to be high irrespective of the density. The experiment is still on-going, hence no recommendation for the optimum density for both containers can be made at this time. The optimum density will be determined once the abalone attain the 3.0 cm SL.

A Focus Group Discussion (FGD) was conducted with the officers and members of the Sitio Lo-oc Small Fisherfolk Association (SILOSFA) to know about the history, activities conducted, challenges faced and the activities currently implemented by the organization. Seven members (4 officers and 3 members) participated. The FGD guide was divided into three themes: socio-economic/ demographic profile of the participants, structure and membership process and the perceived capacity of members/officers in carrying out their responsibilities.

The average age of the participants was 52, 4 were married and majority were fishers (one was a food vendor). The average number of children for those who were married was 5. The average household size of the participants was 4. The current set of officers was elected in 2011 and was tasked to assist in the management of the Marine Protected Area encompassing four sitios (districts) of the barangay. The organization has currently 29 active members. When asked to assess the effectiveness of the officers in carrying out their responsibilities, the participants gave the highest rating to the Chairman and Vice Chairman (9.3 out of 10) while the scores of 8.4 and 5 were given to the Board of Directors and the members, respectively, because of their lack of participation in meetings and other organizational activities. The major challenges faced by the organization were illegal fishing activities and misunderstandings arising from gossips and lack of information about projects. The participants also said that the officers and members usually approach the current Municipal Environment and Natural Resources officer (MENRO) if they need technical assistance.

A survey was conducted to collect baseline information from ninety (90) fishers consisting of members and non-members of the local fishers' organization. The questionnaire captured data on fishing activities, collection, utilization and marketing of sea cucumber and perceived impacts of the sandfish sea ranching project as well as the impacts of the recent typhoon (Typhoon Haiyan) on their household and livelihood activities. An FGD and mapping exercise were also conducted to understand the current situation and needs/problems of the community as bases for planning for appropriate intervention strategies.

After a preliminary assessment of various sites in Panay Island, Batan Bay in New Washington, Aklan was chosen to be the site for the experiment on determining the most efficient culture system for oysters because it is the only site with minimal use of stakes for growing oysters and with a receptive local government willing to provide support for the conduct of the research. A Memorandum of Agreement has since been signed between SEAFDEC/ AQD and the municipality of New Washington, Aklan.

Oyster spats were collected using experimental rafts with different substrates (recycled motorcycle tires and dried oyster shells). The tires were sliced into half and tied to the raft with plastic binder at 15/ raft. Six pcs dried oyster shells were hung on a nylon string at 12 cm apart and tied to the raft at 42 strings/raft.

Collected spats were reared in new culture systems, the pouch and the tray. Based on a one month rearing period, oysters reared in pouches and trays did not significantly differ in growth rates in terms of length and weight at approximately 1 cm and 20 g mo-1, respectively. No clear trend can be observed yet since the experiment has been on-going for only a month.

Mean survival of oysters was significantly higher in pouches (99.82 ± 0.31%) than in trays (97.16 ± 2.04%). The higher mortality rate in trays was not only due to dead oysters but also due to missing oysters which were considered dead. In future grow-out culture activities, trays will be covered to avoid having these losses.

Species with potentials for resource enhancement

The studies on *Tridacna gigas* has been completed in 2013. The stock enhancement of mud crab and the community managed sea ranching and stock release of sandfish *Holothuria scabra* are also underway. These two studies are part of the GOJ-funded initiative under the Sustainable Aquaculture Program and are reported in the GOJ-TF section of this Highlight.

Adapting to climate change (CC)

The changing global weather patterns brought about by increased global emission of carbon dioxide into the atmosphere are predicted to have serious impacts on many life forms on earth. This year alone, an estimated all-time high of 34 billion tons of carbon dioxide emission was already recorded, an increase of 3% compared to last year (European Commission's Joint Research Center). The high level of carbon dioxide in the atmosphere has already resulted in global warming and has changed the chemistry of the world's oceans in ways that are already harming the coral-reef ecosystem and shell-building organisms (IPCC 2007). These changes could lead to broad impacts on marine ecosystems. The extreme weather disturbances, like more frequent and stronger typhoons, long dry spells resulting in droughts, frequent heavy rains resulting in severe flooding, that have been observed in recent years are some of the phenomena that are linked to climate change.

The sustainability of aquaculture will be further challenged in this scenario since the effects of these climatic changes on the aquaculture organisms are largely unknown. The small-scale fish farmers in the region that produce the great bulk of the aquaculture production are particularly vulnerable since they are dependent on aquaculture operations for food and income. Some urgent adaptation measures are therefore required in response to the threats to food and livelihood provision that may arise due to the changing climatic conditions observed around the globe.

The overall goal of the program is to identify the accompanying changes in the environment brought about by the changing climate that may affect the aquaculture sector, prepare the sector to the possible effects that these changes may have on aquaculture operations, minimize and mitigate the adverse impact of climate change in aquaculture, and ensure the continued operations of all aquaculture production system under changing climatic conditions.

The objectives of the program are: (a) to gather scientific information on susceptibilities of various aquaculture species to the combined effects of increasing water temperature and acidity; (b) to gather scientific data on the effect of climate change to production of natural live food for the hatcheries and fishponds; (c) to promote public awareness on the possible effects of climate change to aquaculture activities and to fish farmers; (d) to assist the government agencies in the country and in the region in gathering baseline information on aquaculture areas that are susceptible to climate change effects; (e) to gather scientific information that will serve as basis for the formulation or design of alternative aquaculture systems that are adaptive to climate change; (f) to collaborate with other institutions in the country and in the region in gathering baseline information on the effects of climate change to mangrove and coral reef ecosystems; and (g) to explore potential adaptive measures to mitigate the impacts of climate change to the aquatic system.

Susceptibilities of various aquaculture species to the combined effects of increasing water temperature and acidity

The effects of increasing temperature on the different stages of development of the abalone *Haliotis asinina* were investigated. Very limited information is available on how elevated temperature affects the different developmental stages of tropical marine invertebrates. This was investigated in tropical abalone (*Haliotis asinina*).

Experiments were conducted to expose the different stages of abalone to elevated temperature treatments [control - ambient (28-29°C), 31°C, 33°C].

Embryonic stage was significantly affected by very high temperature. When newly fertilized eggs were incubated at 33°C, very few embryos developed and hatching rate was very low. Hatching rate was only 20% at 33°C while 86% at ambient temperature. Larval survival and settlement success were also adversely affected by elevated temperature. Survival was significantly lower in larvae reared at 31°C and 33°C compared with ambient temperature. Juvenile abalone were more resilient to the effects of elevated temperature. When juvenile abalone (3 cm) were reared in temperatures 31 and 33°C for 1 month, the growth and survival were not significantly affected.

Elevated temperature has an adverse effect on abalone breeders. Mature females were very sensitive to very high temperature. After 45 days of exposure to 33°C all the female breeders died, while only 10 % of the male breeders survived until 60 days. Furthermore, there was a significant reduction in growth and feeding rates of breeders reared at elevated temperature. Their gonads have also regressed. After 3 months, there were 80% survival in breeders reared at ambient temperature, while 50% in breeders reared at 31°C and none left in 33°C.

The above results indicated that developing or early stages and spawners were very sensitive to the effects of elevated temperature. These stages have a very narrow thermal tolerance compared with the juvenile stage.

To further investigate the effect of acidic conditions and elevated temperature on marine organisms, experiments on the effect of climate change on coral growth and survival were conducted. Corals were exposed to elevated temperature and acidic conditions in the laboratory. In this study, coral fragments (*Porites* sp.) were collected and kept alive in a tank with a continuous flow of fresh sand-filtered seawater. Treatments were: temperature [ambient temp: 28-29 (control) and 31°C]; pH [>8.0 (control) and 7.5]. Acidic condition (pH <8.0) was achieved by bubbling of CO_2 gas to the culture water. Low survival and reduction in growth was observed in corals exposed to acidic condition and higher temperature. This shows that climate change can adversely affect the coral reef population that will eventually result in the reduction of the coral reef productivity.

Meeting social and economic challenges in aquaculture (MSECAP)

The R&D activities of the MSECAP intend to address the four categories of social and economic problems in aquaculture discussed and adopted for development of action plans during the *ASEAN-SEAFDEC Fish for the People Conference*. These issues define the scope and coverage of the MSECAP as follows: (i) enhancing the role of aquaculture in addressing food, income and livelihood security through improved governance, multi-agency collaboration, and comprehensive and inter-disciplinary approaches; (ii) promoting sustainable aquaculture through enabling polices that support the management of natural and environmental resources; (iii) enabling mechanisms, institutions and infrastructure to encourage adoption of better aquaculture practices; (iv) understanding and improving linkages from production to marketing and trade of fishery products to support small and medium enterprise (SME) development; and (v) strengthening the capacity of aquaculture stakeholders by mainstreaming specific rural and peri-urban aquaculture programs and policies in local, national and international development programs.

The goal of the program is to develop and implement social and economic strategies in aquaculture and resource management to secure food and income through stakeholder collaboration.

The objectives of the program is to respond to the specific recommendations for meeting the social and economic challenges in aquaculture identified and adopted during the *ASEAN-SEAFDEC Fish for the People Conference* in June 2011. These include: (1) prioritizing collaborative R&D in aquaculture in the region to have a clear regional assessment and understanding of the role of aquaculture in poverty alleviation and provide basis for policy formulation; (2) allocating R&D resources to address emerging issues on the impacts of climate change and global trade on aquaculture with emphasis on small-holder fish farmers; and (3) enhancing multi-agency collaboration, sharing of information and resources between and among SEAFDEC and its Member Countries and other organizations in addressing the common problems of alleviating the socioeconomic conditions of the poor sector of region.

On-farm demonstration and verification of giant freshwater prawn (GFP) grow-out with tilapia to fisherfolks in Laguna Lake. Photo 1) researchers and technician guide fisherfolks in their parallel culture run; 2) GFP co-culture with tilapia; and 3) discussions between LGU, fisherfolks and SEAFDEC/AQD researchers to discuss technology adoption action plans

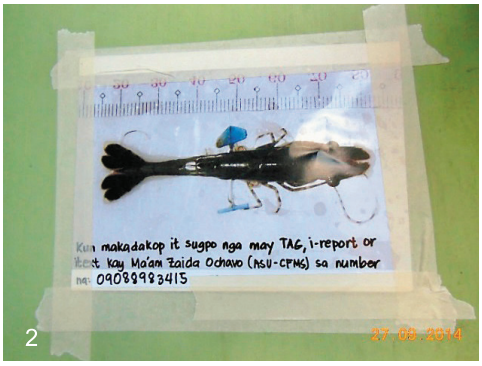
Community-based strategies for managing released abalone stocks in Sagay Marine Reserve, Negros Occidental. Photo 1) Location of release demonstration site in the inter-tidal flats of Brgy Molocaboc (inset, abalone sample 8.3cm shell length, 136g body weight); 2) the stock enhancement signage remains afloat in 2014 since first release in June 2011 and survived super typhoon Haiyan in 2013 symbolizing resilience of the community-based collaboration; 3) meeting between the Sagay City Mayor, SMR and SEAFDEC/AQD; and 4) active BFARMC fisherfolk and the researchers.

Prioritizing collaborative R&D in aquaculture in the region

The number of studies under MSECAP that engage different modalities of collaboration, demonstration and participation of small-scale aquaculture technology adoptors in the Philippines increased from three to seven studies in 2014. For freshwater aquaculture, a third on-farm demo-run of grow-out culture of giant freshwater prawn (GFP), *Macrobrachium rosenbergii*, in polyculture with tilapia in net cages in Laguna Lake was conducted.

The studies that addressed this objective demonstrated that new aquaculture technologies such as the grow-out culture of GFP in cages in Laguna Lake could be integrated either with existing tilapia fingerling or grow-out production that already provide modest incomes to fish farmers. The tilapia growers reiterated that they would not give-up tilapia fingerling production and grow-out culture which had been their year-round livelihood due to continuous consumer demand. They agreed to adopt GFP culture if in polyculture with tilapia.

The stock enhancement of marine species such as abalone *Haliotis asinina* and tiger prawn *Penaeus monodon* were continued through community-based strategies in Sagay Marine Reserve (SMR) in Negros Occidental and New Washington Tributaries in Aklan, respectively. The release strategies and the performance of the hatchery-bred juveniles of abalone and



Area capability development through community-based stock enhancement of shrimps in New Washington, Aklan, Philippines. 1) Sampling of shrimps during nursery culture, 2) tagged shrimps released, 3) abandoned pond in mangrove area serving as intermediate nursery shrimps, 4) fishing gears that are likely to catch released shrimps, 5) fishers and household members participate in mapping local resources in relation to stock enhancement, and 6) fisherfolk attend the IEC to motivate cooperation in fisheries management

Advancing integrated multi-trophic aquaculture (IMTA) in milkfish mariculture through sustainable livelihoods approach. Photo 1) Excess feeds in milkfish mariculture in Lingayen Gulf in Region 1 can be utilized through IMTA, 2) measuring environmental parameters in cages in Dawis Strait in Bohol in Region 7, and 3) milkfish harvest in cages in Malalag Bay in Davao del Sur in Region 11

shrimp, together with the participation and cooperation of the fisherfolk in community-based activities are being monitored. Ten batches of hatchery-bred abalone juveniles, altogether 10,000 pieces, have been released since 2011 to 2014 in the stock enhancement demonstration site in Brgy Molocaboc, a populated fishing community within the SMR. Monthly monitoring of the community-based stock enhancement of abalones showed that they established in the release demo-site in coral areas in the intertidal flats of Barangay Molocaboc within the SMR. The fisherfolk organization already derive cash benefit from the "stock-protect-regulated harvesting" protocol that promote the 6cm shell length catch-size regulation for abalone.

In Aklan, the fourth run and release of tiger shrimps in in the New Washington Tributaries is on-going in collaboration with the LGU, a local fisherfolk organization and local fisheries university. Information, education and communication activities continue to support fisheries regulations and policies to sustain benefits from stock enhancement in both provinces of Negros Occidental and Aklan. The survival rate of tiger shrimps is now at 44% during the intermediate culture phase. The fisherfolk also reported that tagged tiger shrimps released for stock enhancement have been found in their catch. The number of community members interested in participating in the project and the number of fisherfolk PSFA members also increased as they expect to benefit from increased catch of shrimps in future releases. These abalone and shrimp stock enhancement projects continue to demonstrate the important role of aquaculture in fisheries management and developing supplemental livelihood opportunities for the fisherfolk.

Allocating R&D resources to address emerging issues

Due to climate change and global trade, the potential applications of income-generating but eco-friendly culture technologies such as the integrated multi-trophic aquaculture (IMTA) in milkfish mariculture is being evaluated in a survey of milkfish cage and pen owners in three regional sites, namely Lingayen Gulf in Region 1, Bohol Strait in Region 7 and Davao Gulf in Region 11. The survey tended to indicate that the IMTA concept is acceptable among mariculture operators due to ecological and economic benefits, but cage design and sources of seeds of co-culture species such as sea cucumber, oyster, mussel and seaweeds are major concerns.

Enhancing multi-agency collaboration at the local and international levels

The MSECAP program likewise collaborated and contributed to the FAO-AFSPAN project that investigates the role of aquaculture in poverty alleviation, food security and nutrition improvement.

Most recently, MSECAP also collaborated with a local private company to conduct a market, industry and consumption study of selected high-value indigenous species. The participation in the global FAO-AFSPAN project through surveys and review studies have provided updated primary and secondary data for evaluating the contribution of aquaculture in poverty alleviation, food security and promotion of improved nutrition in low-income and developing countries.

SEAFDEC/AQD welcomes its new Deputy Chief

On April 1, 2014, SEAFDEC/AQD welcomed its new Deputy Chief, Dr. Takuro Shibuno. Dr. Shibuno, 56 years old, is a scientist at the FRA-NRIA of Japan (Fisheries Research Agency – National Research Institute of Aquaculture) with expertise on ecology of reef fishes and conservation ecology of coral reefs & rocky reefs. Before being seconded to AQD, he headed NRIA's coastal ecosystem group and worked on the changes in biodiversity of artificial reefs and the impact of ocean acidification on early development of the economically valuable gastropod *Turbo cornutus*. As author and co-author of many science papers, Dr. Shibuno has published in the Journal of Fish Biology, Marine Biology, Fisheries Science, Coastal Marine Science, Crustacean Research, among others. He has also served as head of NRIA's project management division for four years. Dr. Shibuno is married with a 22-year old son and likes scuba diving and lawn tennis.

Promotion of sustainable and region-oriented aquaculture practices

Since January 2010, SEAFDEC/AQD has been continuously implementing a regional program entitled "*Promotion of sustainable aquaculture and resource enhancement in Southeast Asia*" through trust funds provided by the Government of Japan under the ASEAN-SEAFDEC Fisheries Consultative Group Program. In collaboration with several research partners in the ASEAN region, the program implements the following four projects: (I) Promotion of sustainable and region-oriented aquaculture practices (Sustainable Aquaculture); (II) Resource enhancement of internationally threatened and over-exploited species in Southeast Asia through stock release (Resource Enhancement); (III) Accelerating information dissemination and capacity building in fish health management in Southeast Asia (Fish Health); and (IV) Food safety of aquaculture products in Southeast Asia (Food Safety).

Sustainable Aquaculture

The objectives of this project are to: 1) establish reliable mass seed production techniques for genetically improved strains of commercially important species through selective breeding and to develop hatchery technology for newly emerging species for aquaculture; 2) develop environment-friendly and cost-effective practical feeds using ingredients available in the region and establish guidelines on feeding management for sustainable aquaculture; 3) develop farming management strategies that eliminate the risk factors through epidemiological and environmental approaches to prevent and control diseases; and 4) verify and disseminate the project achievements especially in the lesser developed countries in the region through demonstration, training, lecture/seminar and publication activities.

Selective breeding of black tiger shrimp *Penaeus monodon*

The first batch of wild broodstock used as base population produced nauplii which were reared in tanks, but showed slow growth and only one batch of nauplii was produced from these F_1. However, this batch of nauplii (F_2) tested positive for Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV) when analyzed at the postlarval stage. A second batch of wild broodstock was collected from Masbate, Negros, Bohol, and Davao and these resulted in production of nauplii (F_1) which all came out negative for both White Spot Syndrome Virus (WSSV) and IHHNV. The larger postlarvae and juveniles were grown in biosecure ponds to broodstock size. F_1 broodstock were induced to mature, and although several maturations were observed, many resulted in regressed ovaries. Comparison of reproductive performance between F_1 females stocked with wild-sourced male broodstock and those stocked with F_1 males showed that two maturations and production of one batch of nauplii resulted for each.

Genetic improvement of giant freshwater prawn *Macrobrachium rosenbergii*

This study assessed the effectiveness of broodstock management schemes for improved growth and reproductive performance of the giant freshwater prawn. The following techniques have been tried to minimize inbreeding in hatchery stocks: (a) use of new spawners from the original broodstock source (Calumpit, Bulacan, Philippines) to replenish hatchery breeders; (b) reciprocal mating using stocks from a singular source but obtained at different years; and (c) procurement of new stock from a geographically and presumably genetically different source (Pampanga River, Philippines). Refreshing the old pool of hatchery broodstock with stocks from the same source has shown advantage in terms of growth while use of reciprocal mating as a method have shown gains in growth performance only for one generation of prawn production. When growth assessment runs of five prawn stocks namely Old Calumpit (OC) purebred, New Calumpit (NC) purebred, NC-OC crossbred, OC-NC crossbred and Pampanga (P) purebred strain were conducted, specific growth rate was highest for the NC-OC crossbreds (2.56% BW gain/day) followed by OC purebreds, OC-NC crossbreds, the Pampanga stock and the NC purebreds. Survival for all stocks after 169 days of culture ranged from 25% to 33% with the OC purebreds having the highest survival. In contrast, in terms of breeding performance, NC purebreds had the highest average number of hatchlings at 363 eggs/g BW female (based on five months of breeding data) while the lowest was that for the OC purebreds.

Development of hatchery techniques for pompano and spotted scat

The optimum hormone concentration to induce spawning in spotted scat was determined in this study. Since 2013 several spawning trials have been done, the female spawners were able to respond to 2 successive injections of LHRH-a at 24h interval, however male fish were not able to fertilize the eggs. In 2014, only two spawning trials were conducted because all the mature breeders died in May due to very low levels of dissolved oxygen at the DBS ponds. The fish that survived were still very young.

The use of probiotic in the hatchery culture of pompano was conducted. Probiotic added to live food organism as larval food for pompano promoted better assimilation of nutrients as indicated by the increase

in the enzyme activities in the larvae that resulted in better growth, survival and tolerance to hypoxic condition.

Development of efficient and low-pollution diets for grow-out and broodstock

A 180-day feeding experiment was conducted under laboratory conditions to evaluate the potential use of cowpea meal (CPM) as a protein source for *Macrobrachium rosenbergii* broodstock. Four isocaloric and isonitrogenous (approximately 40% crude protein) experimental diets were formulated by replacing levels of fishmeal (FM) with cowpea meal (CPM at 0, 10, 20 and 30%) and their effects on reproductive performance and offspring quality were assessed. Moulting and spawning frequency did not differ between treatments. However, spawn performance in terms of the total number of spawns was higher at 30% CPM while egg production per female was better at 0% CPM. Each female broodstock produced approximately 4,750 to 6,549 larvae per hatching event, the highest of which was found in 30% CPM level. Differences in offspring quality parameters however, were not significant among treatments.

Disease tolerant and sustainable aquaculture environment

Diseases of known and unknown aetiology continue to plague the shrimp industry. The best way to avoid any disease outbreak is to make the shrimp tolerant to pathogens by minimizing stress. Optimizing environmental conditions, specifically pond water quality, could prevent stressful conditions. Water quality is affected by pond management techniques. This project aims to verify management schemes that will prevent or mitigate disease occurrence in commercial ponds. Application of organic fertilizer (a mixture of bokashi, chicken manure, fish amino acid, seaweed extract, and molasses) provided an abundant supply of natural food in the pond. The average body weight of *P. monodon* at DOC 78 was 16g. No WSSV outbreak occurred even after infection at DOC 61.

Resource Enhancement

The objectives of this project are to: 1) establish mass production technology and broodstock management, and to develop methodology of stock enhancement of internationally threatened species (species listed in CITES); 2) establish release strategies of regionally over-exploited species and verify the effectiveness of community managed sea ranching and socioeconomic strategies; 3) establish adaptive measures supporting resource enhancement for a changing environment; and 4) disseminate and demonstrate resource enhancement practices.

Stock enhancement of seahorses *Hippocampus comes*

Stock enhancement of seahorses needs to be carefully managed through preparatory activities such as evaluation of marking and tagging methods in order to monitor the released animals and establish the impact of stocking. The viability of tagging techniques for seahorses using the Visible Implant Elastomer (VIE) was evaluated using three size groups of seahorses (size A – 6.25 cm SH, 0.75 g; size B – 9.2 cm, 2.4 g and size C – 11.7 cm, 5.75 g) at 20 animals per treatment using yellow, orange and red fluorescent VIE. Another set of 20 animals per three size group served as control. Only red VIE tag was visible under the naked eye (without the aid of ultra violet light), while yellow show the brightest color (score of 3), followed by red (score of 2) and orange (score of 1) under the UV light. Mortality occurred in size A seahorses one day after injection of tag. All size groups increased in stretched height and body weight after 180 days of culture with size A having higher daily growth rates compared to sizes B and C. Tagged size A seahorses (10-15%) have lower survival than the control group (25%) after 180 days of observation. Survival of size B and C seahorses were higher, thus tagging did not have any effect on the survival of bigger size seahorses.

Hatchery produced *H. comes* with size range of 10-13 cm were transported and released in Molocaboc Island in Sagay City, Negros Occidental on 10 December 2014. No mortality was observed after 8 hours of travel. One day after release of the seahorses, recovery of hatchery-reared seahorses was 20%. A total of 22 wild seahorses were sampled for stretched height, body weight and gonad development.

Stock enhancement of Napoleon wrasse *Cheilinus undulatus*

Previous site assessment activities revealed that Danajon reef in Bohol Island was known to harbour a number of Napoleon wrasse. In order to establish proper protocols for future stock enhancement program, two baseline surveys were done to gather information on the Napoleon wrasse population in Bohol and to determine the awareness of the local people on environmental conservation and protection. There were 56 residents of Brgy. Mantatao who were interviewed in each survey. Eighty percent (80%) of the respondents were directly harvesting aquatic resources from the sea as their primary livelihood. Almost all of them can identify the Napoleon wrasse from the picture included in the questionnaire. However, the scanty answers on items regarding stock enhancement showed low awareness of people and absence of such programs in the area.

Stock enhancement of mud crabs *Scylla* spp.

This study aims to address the declining mud crab population in mangrove areas by establishing stock enhancement technology using SEAFDEC hatchery-bred juveniles. Sampling of mud crabs from Brgy. Rojas, Ajuy, Iloilo was done by fishers contracted to undertake standardized fishing every spring tide. All crabs caught were identified to species level, and measured for internal carapace width (mm CW) and body weight (g BW). Prior to release, crabs were tagged by size batches in the basal muscle of the fourth pereiopod on the left side by injecting a magnetic decimal sequential coded microwire tag. From January to June 2014, sampling of mud crabs after release of hatchery crabs on November 2013 was done 12 times. A total of 699 mud crabs have been collected

from 12 samplings, of which 123 were recaptured released crabs. Recaptured crabs comprised 17.45% of the total mud crabs caught in 6 months and represented 15.35% of the total crabs released in the mangroves of Brgy. Rojas, Ajuy, Iloilo. Growth rate of wild *S. olivacea* tagged and released in the mangroves ranged 0.18-2.04 cm CW mo^{-1} and 1.00-82.44 g BW mo^{-1} with a mean of 1.01±0.03 cm and 33.07±1.40 g mo^{-1}, respectively. Mud crabs released in the mangroves increased yield and catch per unit effort, CPUE, of fishers.

Socioeconomic analysis and identification of strategies for managing released stocks of abalone

This five-year study (2010-2014) aims to identify and implement strategies for managing released stocks of abalone in the Sagay Marine Reserve (SMR) in Negros Occidental and contribute to the development of policies for managing abalone fisheries in similar areas in the Philippines and SEA. A total of 14,020 hatchery-bred abalone juveniles with 2.5 mean shell length (SL) have been released in 12 batches since the first release in June 2011 to most recent in September 2014. A total of 2,247 pieces or 148 kg have been harvested periodically since August 2012, valued at Php 42,738 credited to the BFARMC income. In 2014, the "stock-protect-partial harvest" protocol continues, surviving the adverse disturbances of typhoon Haiyan in November 2013. On November 2014, a total of 160 fisherfolk were interviewed to assess their experiences and feedback on the stock enhancement conducted in Brgy Molocaboc. The respondents overall appreciated the positive impact of stock enhancement in terms of rebuilding local abalone fisheries in the long-term. They also reported intention to adopt the "stock-protect-partial harvest protocol" as a group activity, however, they reported limitations in term of access to suitable site and sources of juveniles.

Adaptive measures for coral replenishment: Baseline study for diagnosis of coral reef health & establishment of coral replenishment technology

The function of photosynthesis was proved to be largely damaged by the synergistic effect of acidification and warming through analyses of the photosynthesis and metabolism ratio, since no significant decreases were detected by a single physical driver either of acidification or warming. This exacerbation was also demonstrated in the disappearance of correlation between respiration with photosynthesis functions in contrast to the positive correlations between them when confirmed in both single effects of acidification and warming.

Fish Health

The objectives of this project are to: 1) accelerate awareness about fish health management in resource-deprived countries through industry-wide capacity building; 2) guarantee food safety and sustainable production through innovative research; and 3) disseminate output of the project.

Status and needs of primary aquatic animal health care in small-scale aquaculture

A survey was conducted in Myanmar, Philippines, Lao PDR, and Cambodia to identify the gaps and needs in order to equip small farmers and other industry stakeholders with capability to monitor disease, identify and prioritize important small-scale aquaculture commodities, conduct audits of selected farms to identify key issues affecting fish health and production, boost staff capability as well as translate learning materials, conduct on-site and other training courses and guided research and information dissemination.

Compared with those in Myanmar and the Philippines, small-scale farmers in Lao PDR and Cambodia had a lower level of awareness of fish health management and food safety, and other issues affecting fish production in ponds.

There is a need to strengthen the expertise of government fish health staff in Myanmar, the Philippines, Lao PDR, and Cambodia in order to affectively disseminate available information on fish health management.

Surveillance and training of fishborne zoonotic parasites of commercially important freshwater fish in some Southeast Asian countries

In 2014, tissue samples of freshwater fish species were collected from some provinces of Region XIII-Caraga, Philippines. A total of 560 freshwater fish species including *Clarias* sp., *Ophicephalus* sp., *Tilapia* sp., *Cyprinus* sp., *Glossogobius giuris, Hypseoleotris agilis, Mugil cephalus,* and *Glossigobius celebius* were collected from earthen ponds and lake. Only *H. agilis* from Lake Mainit, Surigao del Norte were positive for the presence of fishborne zoonotic metacercariae, specifically *Clonorchis* sp., with a prevalence rate of 14.28%. In addition, a total of 300 freshwater fish of 2 species (*Tilapia* sp. and *Cyprinus* sp.) were collected from La Union, Ilocos Sur and Ilocos Norte. Results showed that no metacercariae were detected in all fish examined. A follow-up survey of fish-borne zoonotic trematode (FZT) metacercariae in farmed fish was carried out in selected fish farms in Vientiane and Luang Prabang, Lao PDR. A total of 460 fish involving five species was tested, and results showed no metacercariae. However, monitoring is a must because conditions can change rapidly and food safety is a legitimate concern of Southeast Asian countries.

On-site Basic Training on Freshwater Fish Health Management

In conjunction with the primary objective of the Government of Japan Trust Fund V Fish Disease Project, i.e. to accelerate awareness on fish health management in resource-deprived countries through industry-wide capacity building, the on-site basic training on freshwater fish health management was conducted in three SEAFDEC Member countries (Myanmar, Lao PDR and Cambodia) from 2011 to 2013. For Lao PDR, this was carried out in collaboration with Department of Livestock and Fisheries, Ministry of Agriculture and Forestry, Lao PDR from

11 to 14 October 2011 and followed up from 05-08 December 2014. The primary target of the 2014 on-site course was to enhance the skills of the previously trained fish health workers on detection of freshwater fish zoonotic parasites collected from various Vientiane sites. The course was held at Namxouang Aquaculture Development Center, Department of Livestock and Fisheries, Ministyry of Agriculture and Fisheries, Lao PDR with funding support from SEAFDEC/AQD and GOJ-TF.

Molecular diagnosis and prevention of viruses in economically-important fish and shrimp

The plasmid of important shrimp viruses (WSSV, IHHNV, IMNV and TSV) and fish viruses (KHV and VNN) were already developed. These plasmids were used in the optimization of the q-PCR and also served as positive controls for conventional Nested PCR. Optimization of q-PCR protocols for WSSV, IHHNV, IMNV, TSV, KHV and VNN were already completed. Local WSSV isolate was already tissue-passed thrice and the infected tissue material is being used in the on-going preliminary infection experiment in determining the threshold level of the virus. Developed plasmid positive controls and the optimized q-PCR protocols can now be used in the Diagnostic Services of Fish Health Section in the detection of shrimp and fish viruses. LAMP assay for WSSV, IHHNV, IMNV, TSV, RSIV, KHV and VNN were also optimized and will serve as an alternative detection method that is cheap, rapid, sensitive and specific and can be used on-site in the farm.

Establishment of an immunization regimen for the prevention of viral nervous necrosis (VNN) in high value marine broodfish

Outbreaks of viral nervous necrosis (VNN) in pompano (*Trachinotus blochii*) larvae remain as a major deterrent in the successful production of this fish species in hatcheries. The current data support the idea that annual booster vaccination of pompano broodstocks reared in floating net cages could immunogenically mount the production of NNV-neutralizing antibodies in these fish thereby conferring protection against horizontal transmission of the NNV via contaminated feed or water and consequential vertical transmission of the NNV to their offspring. The current data importantly demonstrate the practicality of maintaining VNN-specific-free pompano broodstocks reared in floating net cages in the open sea through vaccination regimen.

Establishment of novel prophylactic and therapeutic methods for the prevention of viral infections in commercially important maricultured fish

The *in vitro* susceptibility of fish pathogens such as *Aeromonas hydrophila* (4), *A. sobria* (1), *Pseudomonas putida* (1), *Vibrio harveyi* (2) and *V. coralliilyticus* (6) to *Ulva pertusa* crude ethanolic extract was examined using the cup-well method. *U. pertusa* crude ethanolic extract exhibited antibacterial activity against *A. hydrophila* (only 2 out of 4 isolates examined) as indicated by the absence of bacterial growth (clear zone) measuring 20 and 10 mm around the wells containing 100 and 50 mg of the extracts, respectively, but not at lower concentrations of the extract. Also, absence of *A. sobria* growth was only noted around the wells containing 100 mg (18 mm) and 50 mg (10 mm) of the extract. In addition, *Pseudomonas putida* was minimally susceptible to the extract at 100 mg (13 mm) but not at ≤50 mg. On the contrary, all *V. harveyi* and *V. coralliilyticus* strains exposed to the highest concentration of the extract were resistant.

Evaluation of carriers for practical delivery of vaccines to shrimp and other crustaceans

Trials were conducted to test various carriers for delivery of recombinant VP28 sub-unit 'vaccine' to juvenile shrimp, *Penaeus monodon*. Chitosan and alginate microparticles were subsequently prepared and used to encapsulate and deliver the vaccine thru the feed to improve its efficacy. Upon challenge with WSSV, the vaccine entrapped in chitosan showed significantly highest survival among the test groups although the RPS was below 50%. The complexes were then incorporated to the feed and administered orally to the shrimp. The second WSSV challenge test showed that survival was again highest in IB+chitosan (65.3%), followed by IB+alginate (61.9%), naked IB (50%), inactivated recombinant bacteria (25.9%), and lowest with the unvaccinated group (0%). There were no significant differences in survival between IB+chitosan and IB+alginate, but these groups were higher by at least 24% compared to naked IB, inactivated recombinant bacteria and the unvaccinated group.

Parasitic and shell diseases of abalone *Haliotis asinina* in the Philippines

This study describes the first screening of *Haliotis asinina* for the presence of parasites and shell diseases in the Philippines. Macroscopical and histological analyses were carried out in the abalone's shell and tissues. Results revealed that hatchery-reared abalone are routinely infested by shell-infesting polychaete worm belonging to the family *Dorvilleidae* (prevalence, 35%). Nematodes were also found with prevalence of 11%. Gross examination of wild-caught abalones revealed shell damage associated with burrowing polychaetes, belonging to the family Dorveillidae (prevalence, 10%). Histological examination showed that the overall prevalence of the various parasites were very variable in hatchery-reared abalone and wild-caught abalone. A metazoan parasite (*Tylocephalum* sp.) was detected in hatchery-bred abalone and from the wild (7% and 3% prevalence). Ciliates were detected on the hatchery and wild stock representing a prevalence of 36% and 6% respectively. For all condition indices, affected abalone consistently yielded significantly lower condition indices than unaffected individuals with shell-boring polychaetes. The infectivity rate of shell-boring polychaetes in naturally-infected to healthy abalone was 60%. Results showed that at 96h post treatment, 70% abalone mortality was observed in those exposed to 50 ppm mebendazole; but shell-boring polychaetes mortality approached 100%.

Food Safety

The objectives of this projects are to: 1) contribute in the establishment of guidelines on the production of safe aquaculture products from Southeast Asia; 2) determine the presence and levels of commonly used chemicals in aquaculture in aquaculture products such as fish and shrimps; 3) compile and disseminate SEAFDEC guidelines on the use of antibiotics and chemicals in aquaculture to the ASEAN region; and 4) implement training course/workshop to promote food safety awareness in the ASEAN region.

Withdrawal period of antibiotics in fish species cultured in the tropics: Pompano *(Trachinotus blochii)*

The withdrawal period of oxytetracycline (OTC) and oxolinic acid (OXA) in pompano (*Trachinotus blochii*) was determined following oral administration. Fish were fed OTC or OXA medicated diet (75mg/kg fish/day) 3 times a day for 10 successive days at 2% body weight and thereafter switched to regular diet for 45 days. Muscle and blood samples were taken at regular intervals during and after cessation of medication. OTC and OXA residues in the muscle and blood were analysed using the high performance liquid chromatography (HPLC). Withdrawal period at 30°C for OTC in pompano muscle was 19 days (570 degree-days) and 17 days (510 degree-days) in the blood. Withdrawal period at 30°C for OXA in pompano muscle was 5 days (150 degree days) and 2.5 days in the blood (75 degree days).

Surveillance of Chemical Contaminants in Aquaculture Products and Feeds: Micro-organism method for the detection of oxolinic acid (OXA) and oxytetracycline (OTC) in fish muscles

Antibiotics are used in aquaculture to prevent and treat diseases that affect farmed shrimp and fish. The indiscriminate use of antibiotics could lead to drug resistant strain and multiple antibiotic resistances in bacteria. HPLC is the method of choice for the routine analysis of antibiotic detection. However, antibiotic detection using HPLC requires equipment that is not affordable to small laboratories and skilled chemists/ technicians are needed to do the analysis. Therefore, an alternative method of antibiotic detection that is easy and cost effective is needed. This study aimed at developing such a procedure. Results indicated that the microbiological assays could be used to detect OXA and OTC, but lacked the sensitivity required for regulatory purposes.

Investigation of the Situation of Antibiotics/Chemical Usage and Regulations in Aquaculture towards the establishment of guidelines for the appropriate administration and regulation of chemical use

AQD publication "Important Findings and Recommendations on Chemical Use in Aquaculture in Southeast Asia" (by Dr. Coloso, Dr. Catacutan and Ms. Arnaiz) was finalized for the SEAFDEC member countries by filling the gaps of the ASEAN guidelines.

Training fellowships under GoJ-TF

The GoJ-TF granted 15 fellowships to fishfarmers and government extension workers from SEAFDEC member countries in four of the 28 training courses or sessions organized by AQD in 2014:

Training course	Fellowship granted (number of trainees)
Abalone hatchery and grow-out	Cambodia (1), Philippines (1), and Viet Nam (1)
Marine fish hatchery	Cambodia (1), Brunei (1), Thailand (1), and Philippines (1)
Community-based freshwater aquaculture for remote rural areas in Southeast Asia	Brunei (1), Cambodia (1), Indonesia (1), Malaysia (1), Lao PDR (1), and Myanmar (1)
Distance Learning Course on Principles of Health Management in Aquaculture (AquaHealth Online)	Lao PDR (1), Philippines (1)

Regular and specialized training

The Training and Information Division (TID) of AQD continued to build the capability of fishfarmers and other stakeholders in the industry and the academe on sustainable aquaculture technologies through training, internship and research mentorship.

For 2014, TID conducted 32 sessions of 25 courses that were attended by 313 trainees from 20 countries. TID also continued with its internship program that was availed of by 31 interns, while student on-the-job training was availed of by 221 students from 22 schools from all over the Philippines.

Special Internship on Marine Fish & Abalone Culture (Tigbauan Main Station [TMS], Tigbauan, Iloilo), 28 Jan–01 Feb 2014. This is a specialized internship course requested by the Western Philippine University of Palawan attended by 24 students and two faculty members. The course was tailor-made to provide the students and faculty with theoretical and practical skills on the broodstock management, spawning, larval rearing, and nursery of abalone and marine finfishes.

Training Course on Freshwater Prawn Hatchery and Grow-out Operations (Binangonan Freshwater Station, Tapao Point, Binangonan, Rizal), first session: 17-21 Feb 2014; second session: 5-9 May 2014; third session: 8-12 Sept 2014. A total of 28 participants completed the three sessions of this course. They had lectures and praticals on the biology, hatchery, larval and grow-out rearing techniques of the freshwater prawn. They also had lectures on sustainable management of farmed commodities and aquaculture economics.

Training Course on Mangrove Conservation, Management & Rehabilitation (TMS, Tigbauan, Iloilo), 10-14 March 2014. The participants of this course were from people's organizations, eastern Samar local government units and non-government organizations. The course had lectures on mangrove biology and ecology, taxonomy, and community structures with corresponding field work held in Ajuy, Iloilo and Ibajay, Aklan. The training was part of the environmental rehabilitation project with focus on marine protected areas and mangroves, of the Foundation for the Philippine Environment in collaboration with the Zoological Society of London-Philippines and AQD.

Training Course on Tilapia Hatchery and Grow-out Operations (BFS, Tapao Point, Binangonan, Rizal), 10-14 Mar 2014. The 5-day training course provided the participants with technical knowledge and skills on the biology, hatchery, nursery and grow-out culture of tilapia. The specific topics include the breeding, hatchery and nursery techniques, site selection and water quality management, natural food production, design and fabrication of net cages, nutrition and feeding of tilapia and aquaculture economics.

Internship Training Course on Tilapia Hatchery and Grow-out Operations (BFS, Tapao Point, Binangonan, Rizal), 10, 12, & 14 March 2014 and on 23 Oct 2014. These are hands-on training internships on tilapia hatchery and grow-out operations.

Training Course on Capacity Building on Information Dissemination & Data Management (TMS, Tigbauan, Iloilo), 10-18 March 2014. This is a nine-day course on information dissemination and data management requested by NFRDI. The course primarily covered the skill set required of science writers like news writing, design & layout, photography, website & social network mechanics; and accessing published information found in library databases, among others. With AQD providing training mainly on aquaculture technologies, this is the first time that a non-technical course was offered.

Training Course on Feed Formulation & Preparation (TMS, Tigbauan, Iloilo), 19 March–01 April 2014. This specialized course was designed to offer participants theoretical and practical knowledge on sourcing of feed ingredients, the dietary requirements of aquaculture species and the computations involved in the formulation and preparation of feeds.

Training Course on Seaweed (*Kappaphycus*) Farming (TMS, Tigbauan, Iloilo), 19 March–01 April 2014. The seaweed course included lectures on seaweed industry status, biology & taxonomy of seaweeds, nursery & grow-out culture among others. There were also practical activities such as preparation of culture materials & media, production of micropropagules, land-based nursery culture, planting of seaweeds, and preservation & identification of seaweeds

Training on PCR-Based Diagnosis of the Major Viral Diseases of Fish & Crustaceans (TMS, Tigbauan, Iloilo) 07–08 April 2014. The trainees are taught standard DNA-based methods using specific PCR primers and nucleic acid probes for the polymerase chain reaction method of detecting viral pathogens in fish and crustaceans.

Training Course on Sandfish *(H. scabra)* Seed Production, Nursery & Management (TMS, Tigbauan, Iloilo), 22 April–06 May (1st Session) and 07-21 Oct 2014 (2nd Session). A total of 14 trainees in two sessions attended this course. The course included lectures on topics regarding sea

Trainees for "aquafeed formulation & preparation" course at AQD's pilot-scale feed mill with AQD scientist Dr. Veronica Alava (in yellow).

Trainees in "mud crab hatchery, nursery and grow-out operations" course during practical sessions in the hatchery and laboratory.

cucumbers, in particular the sandfish *Holothuria scabra*. The trainees were also trained on the practical activities in sandfish production including larval rearing, stock management, spawning & grow-out techniques.

Special Internship on Abalone Culture (TMS, Tigbauan, Iloilo), 24 April–03 May 2014. The participants were taught how to grow abalone the AQD way, the abalone is stocked in prefabricated, multi-tier plastic trays specifically designed for its culture at stocking densities of 50 to 100 pcs per m2. It is fed seaweed *Gracilaria* every three days for a culture period of 180 days.

Training Course on Mud Crab Hatchery & Nursery Operations (for DOST Collaborators) (TMS, Tigbauan, Iloilo), 28 April–16 May; 12 May–18 June; 17 May–05 June; 22 May–18 June; and 27 May–18 June 2014 (5 sessions). The course had lectures and practical sessions on biology & identification of mud crabs, nursery management, aquasilviculture, crab fattening, soft-shell crab farming, hatchery construction, disease & health management, economic evaluation of hatchery & nursery phases and the culture of microalgae as feed for the larvae.

Training Course on Abalone Hatchery & Grow-out (TMS, Tigbauan, Iloilo), 07–27 May 2014. This is a regular training course on abalone hatchery and grow-out participated by trainees from Cambodia, the Philippines, Singapore and Viet Nam and partly funded by the Government of Japan.

Training Course on Mud Crab Hatchery, Nursery & Grow-out Operations (TMS, Tigbauan, Iloilo), 27 May–18 June (1st Session) and 07-29 Oct 2014 (2nd Session) The course aimed to provide participants with knowledge on the biology of mudcrabs; the basic principles and procedures for managing mature crabs, rearing of zoea to crab instar stage, nursery and grow-out culture of crabs (ponds and pens in mangroves), fattening and soft shell crab farming; the economic feasibility of the various culture systems; and basic concepts of sustainable aquaculture. The course consists of lectures, practical sessions and field trips.

Training Course on Marine Fish Hatchery (TMS, Tigbauan, Iloilo), 18 June–25 July 2014. Nine participants attended the 37-day course on marine fish hatchery at AQD's Tigbauan Main Station. Trainees came from the government & private sectors based in the Philippines (3), Brunei Darussalam (2), Singapore (2), Cambodia (1) and Thailand (1). A series of lectures and practical activities were done to provide the participants with technical knowledge and skills on the biology, broodstock management and spawning & larval rearing of marine fishes such as milkfish, seabass, grouper, snapper, rabbitfish or pompano. Production of natural food for larvae was also part of the course.

Special Internship on Abalone Culture (for the Municipality of Sta. Maria, Romblon) (TMS, Tigbauan, Iloilo), 23-28 June 2014. In the Philippines, abalone harvest from the wild is drastically decreasing most probably due to overfishing and habitat degradation. Thus there is a need for local government units to learn about the breeding and culture of abalone.

Special Internship Mud Crab Nursery (for DOST Collaborators) (Dumangas Brackishwater Station, Dumangas, Iloilo), 16-18 July 2014. The nursery phase of mud crab aquaculture is an industry in itself. This short course was tailored to the needs of DOST collaborators on the theory and practice of rearing mud crab instars to marketable crablets.

Special Internship on Marine Fish Culture (Milkfish & Grouper) (Romblon) (TMS, Tigbauan, Iloilo), 04-29 Aug 2014. Five participants from the local provincial government of Romblon attended this special course. The training consisted of lectures and practicals on the reproductive biology, spawning and larval rearing of milkfish and grouper.

Training Course on Catfish Seed Production (TMS, Tigbauan, Iloilo), 18-22 Aug and (SEAFDEC/AQD, Binangonan, Rizal), 13-17 Oct 2014. The first session was participated by nine trainees from the private sector from the Philippines while the second session was attended by eight participants from Brunei Darussalam, Sudan and the Philippines. The trainees came from various disciplines such as medicine, engineering, education, and fisheries and aquaculture. The 5-day training course provided the participants with technical knowledge and skills on the biology, hatchery, nursery and grow-out culture of catfishes. The specific topics include the breeding, hatchery and nursery techniques, site selection and water quality management, natural food production, design and fabrication of net cages, nutrition and feeding of catfish, innovative techniques for improved survival of catfishes in the grow-out phase and aquaculture economics. Each lecture was followed by a practical session.

Training Course on Feed Formulation & Feed Evaluation of Aquaculture Species (TMS, Tigbauan, Iloilo), 01-05 Sept 2014. Due to the insufficient number of adequately trained researchers in aquaculture nutrition, the need of upgrading the researchers' technical skills in this area is vital. Hence, the DOST's Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development (DOST-PCAARRD) partnered with AQD to conduct a course on "feed formulation and feed evaluation for aquaculture species". The training provides the participants (25 researchers from 10 colleges and universities) with

the knowledge on nutrient requirements, feed preparation & other practical tools in feed evaluation. Also included in the course are lectures on feeding management and economics. A roster of top-caliber resource persons coming from AQD, University of the Philippines-Visayas and DOST-PCAARRD handled the lectures and practical exercises of this five-day course.

Training Course on Disease Detection in Marine Fish and Crustaceans (For a KSA National) (TMS, Tigbauan, Iloilo), 01-09 Sept 2014. One government-funded intern from Saudi Arabia attended this course that was designed to provide the knowledge on the methods of detection, occurrence and surveillance of the spread of diseases as well as skills in the prevention, treatment and control. The exercises spanned from the detection of major viral & bacterial diseases to histological techniques.

Training Course on Aquaculture Technologies *(Marine Fish Nursery & Grow-out Management) (Shrimp Hatchery & Grow-out Management) (Seaweed Farming)* (For Oman Nationals) (TMS, Tigbauan, Iloilo), 01-12 Sept 2014. Three special courses on marine fish nursery and grow-out culture, shrimp nursery and grow-out culture & seaweed farming were simultaneously conducted and were attended by trainees from Oman (13) & private participants from Malaysia (1) and the USA (1). The training was requested by the Ministry of Agriculture and Fisheries of the Sultanate of Oman to update their technical staff with new operation and management protocols in the production of the aforementioned commodities. Lectures and practical activities were conducted at AQD stations in Tigbauan, Igang and Dumangas. The trainees were taken to a shrimp farm (Negros Occidental), a grouper cage farm (Capiz) and seaweed net cages (Guimaras) to give them the opportunity to interact with private aquaculture practitioners and expose them to the daily practices done at fish farms.

Distance Learning Course on Principles of Health Management in Aquaculture (AquaHealth Online) 08 Sept 2014–22 Feb 2015. This distance training course was attended by 14 participants from various sectors, like, government personnel, the academe and private farm owners from Cambodia (6), Indonesia (1), Lao PDR (1), Philippines (3), Singapore (1), Thailand (1) and the United Kingdom (1). The participants were taught the different aspects of maintaining the health of aquaculture stocks including detection, diagnosis, treatment and prevention of diseases of different etiologies.

Trainees of the "capacity-building on information dissemination and data management" applying their data gathering and photography skills during a study tour at AQD's stations in Dumangas and Igang.

On-Site Training Course on Mud Crab Hatchery & Nursery (ZSCMST, Fort Pilar, Zamboanga City), 22-24 Sept 2014. This course was organized by AQD in collaboration with ZSCMT with funding support from PCAARRD. The three-day course had lectures and practical sessions on mud crab biology, broodstock management & larval rearing, nursery rearing and natural food production. The Industry Strategic S&T Plans (or ISPs) for mud crabs was also presented.

Training Course on Seabass Hatchery Operations (TMS, Tigbauan, Iloilo), 16-28 Oct 2014. The 16-day course included lectures on seabass broodstock management and induced-spawning, larval rearing and nursery culture of seabass in land-based tanks, as well as on the nursery and grow-out culture of sea bass and other marine fishes in cages. Additional lectures comprised the culture of natural food, and the detection and prevention of diseases in sea bass and other marine fishes.

Training Course on Aquatic Microbiology (TMS, Tigbauan, Iloilo), 11-17 Nov 2014. Four veterinarians and one laboratory analyst from the Bureau of Fisheries and Aquatic Resources (BFAR) completed this special training. The five-day course consists of lectures and practical sessions on topics related to testing the pathogenicity of bacterial isolates on fish & crustaceans, characterization and identification of bacteria isolated from diseased fish using conventional methods and a commercial kit, testing the sensitivity of different bacterial pathogens to various drugs, and proper disposal of biohazard specimens and disinfection of experimental facilities. They also visited the Negros Prawn Cooperative Laboratory and other private prawn farms in Negros Occidental.

Training Course on Community-Based Freshwater Aquaculture for Remote Rural Areas of Southeast Asia (BFS, Tapao Point, Binangonan, Rizal), 25 Nov–04 Dec 2014. The 10-day course consisted of lecture sessions, practical activities, and field trips. Lectures ranged from aquatic ecology, design and construction of ponds, pens and cages, diseases of freshwater fishes, natural food production, feed formulation and preparation, food safety in aquaculture, genetics for the sustainable management of farmed tilapia, organizing communities for rural aquaculture, preparatory notes for rapid rural appraisal, policies and governance in rural aquaculture, to business planning and management for sustainable small-scale freshwater farm. Practical activities included sex determination of tilapia, preparation of feed with incorporated male hormone methyltestosterone for tilapia sex reversal; catfish and carp broodstock selection, hormone preparation and injection for induced spawning, stripping of the eggs, fertilization and incubation. Feed preparation, demo on disease diagnosis, and rapid rural appraisal were also part of the practical activities. The trainees had a two-day field trip to the Freshwater Aquaculture Center, College of Fisheries, Central Luzon State University and the Bureau of Fisheries–National Freshwater Fisheries Technology Center in Munoz, Nueva Ecija and a private catfish farm in Sto. Rosario, Pandi, Bulacan.

Information Dissemination

Science Papers

In 2014, SEAFDEC/AQD published a total of 33 scientific papers in ISI-CC indexed journals and conference proceedings . These reports are the outcomes of the long hard work of AQD researchers which will be the foundation of AQD's science-based aquaculture technologies. The 2014 published papers are as follows:

Agbayani RF, Baticados DB, Quinitio ET, Tormon-West DH. 2013. Resiliency of small-holder fishfarmers to climate change and market prices in selected communities in the Philippines. In: Bondad-Reantaso MG, Subasinghe RP (eds.). Enhancing the Contribution of Small-Scale Aquaculture to Food Security, Poverty Alleviation and Socio-Economic Development. Rome: FAO; FAO Fisheries and Aquaculture Proceedings No. 31.; pp. 171-179

Apines-Amar MJS, Andrino KGS, Amar EC, Cadiz RE, Corre VL Jr. 2014. Improved resistance against White Spot Virus (WSV) infection in tiger shrimp, *Penaeus monodon* by combined supplementation of peptidoglycan and mannan oligosaccharide (MOS). Extreme Life, Biospeology & Astrobiology: International Journal of the Bioflux Society 6:1-9

Aralar MLC, Borja AS, Palma AL, Mendoza MM, Ocampo PC, Manalili EV, Darvin LC (eds.). 2013. LAKECON2011: Building on the Pillars of Integrated Lake Basin Management. (Second National Congress on Philippine Lakes) Summary of Proceedings. Los Baños, Laguna: Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development: Department of Science and Technology; 337p

Aya FA, Corpuz MNC, Garcia LMB. 2014. Diet composition, feed preferences and mouth morphology of early stage silver therapon (*Leiopotherapon plumbeus*, Kner 1864) larvae reared in outdoor tanks. Journal of Applied Ichthyology (online first)

Aya FA, Hidaka Y, Kudo I. 2013. Clearance rates and ingestion efficiency of the Japanese scallop *Patinopecten yessoensis*. Plankton Benthos Research 8:1-7

Ayson FG, Sugama K*, Yashiro R*, de Jesus-Ayson EG. 2014. Nursery and grow-out culture of Asian seabass, *Lates calcarifer*, in selected countries in Southeast Asia. In: Jerry DR. (ed.). Biology and Culture of Asian Seabass *Lates calcarifer*, Florida, USA: pp. 273-292

Baticados DB, Agbayani RF, Quinitio ET. 2013. Community-based technology transfer in rural aquaculture: The case of mudcrab *Scylla serrata* nursery in ponds in northern Samar, central Philippines. AMBIO (online first)

Bosma RH, Tendencia EA. 2014. Comparing profits from shrimp aquaculture with and without green-water technology in the Philippines. Journal of Applied Aquaculture 26:263-270

Buen-Ursua SMA, Azuma T, Arai K, Coloso RM. 2014. Improved reproductive performance of tiger tail seahorse, *Hippocampus comes*, by mysid shrimp fed singly or in combination with other natural food. Aquaculture International (online first)

Cuvin-Aralar MLA. 2014. Fish biodiversity and incidence of invasive species in an aquaculture and non-aquaculture site in Laguna de Bay, Philippines. In: Biscarini C, Pierleoni A, Naselli-Flores L. Lakes: The Mirrors of the Earth. Balancing Ecosystem Integrity and Human Wellbeing. Volume 2. Proceedings of the 15th World Lake Conference (Perugia 2014). Umbria Scientific Meeting Association (USMA2007). Italy: Science press; pp. 53-57

Cuvin-Aralar MLA. 2014. Embryonic development of the Caridean prawn *Macrobrachium mammillodactylus* (Crustacea: Decapoda: Palaemonidae). Invertebrate Reproduction & Development 58:306-313

de Jesus-Ayson EG, Ayson FG, Thepot V*. 2014. Early development and seed production of Asian seabass, *Lates calcarifer*. In: Jerry DR. (ed.). Biology and Culture of Asian Seabass *Lates calcarifer*, Florida, USA: CRC Press; pp. 16-30

de Jesus-Ayson EG, Ayson FG. 2014. Reproductive biology of the Asian seabass, *Lates calcarifer*. In: Jerry DR. (ed.). Biology and Culture of Asian Seabass *Lates calcarifer*, Florida, USA: pp. 67-76

de la Peña MR. 2014. Biomass yield of *Isochrysis galbana* (Parke; Clone T-ISO) and growth of *Brachionus rotundiformis* (Tschugunoff) using continuous cultivation method. The Philippine Agricultural Scientist 97:161-170

Denusta PJT, de Jesus-Ayson EGT, Laron MA, Garcia LMB. 2014. Effects of human chorionic gonadotropin (hCG) and handling stress on spermiation of silver perch *Leiopotherapon plumbeus* (Kner, 1864). Journal of Applied Ichthyology 30:448-453

Hagiwara A, Wullur S, Marcial HS, Hirai N, Sakakura Y. 2013. Euryhaline rotifer *Proalessimilis* as initial live food for rearing fish with small mouth. Aquaculture (online first)

Laranja JLQ, Ludevese-Pascual GL, Amar EC, Sorgeloos P, Bossier P, De Schryver P. 2014. Poly-β-hydroxybutyrate (phb) accumulating Bacillus spp. improve the survival, growth and robustness of *Penaeus monodon* (Fabricius, 1798) postlarvae. Veterinary Microbiology 173:310-317.

Lee SY, Primavera JH, Dahdouh-Guebas F, McKee K, Bosire JO, Cannicci S, Diele K, Fromard F, Koedam N, Marchand C, Mendelssohn I, Mukherjee N, Record S. 2014. Ecological role and services of tropical mangrove ecosystems: a reassessment. Global Ecology and Biogeography 23:726-743

Lio-Po GD. Susan Lim LH. 2014. Infectious diseases of warmwater fish in fresh water. In: Woo PTK, Bruno DW (eds.). Diseases and Disorders of Finfish in Cage Culture; 2nd edition. Massachusetts, USA: CABI Publishing; pp. 193-253

Ludevese-Pascual G, dela Peña M, Tornalejo J. 2014. Biomass production, proximate composition and fatty acid profile of the local marine thraustochytrid isolate, *Schizochytrium* sp. LEY7 using low-cost substrates at optimum culture conditions. Aquaculture Research (online first)

Luhan MRJ, Avanceña SS, Mateo JP. 2014. Effect of short-term immersion of *Kappaphycus alvarezii* (Doty) Doty in high nitrogen on the growth, nitrogen assimilation, carrageenan quality, and occurrence of "ice-ice" disease. Journal of Applied Phycology (online first)

Mamauag RE, Ragaza JA, Koshio S, Ishikawa M, Yokoyama S. 2014. Hydrolyzed tuna meat by-product supplement for juvenile red sea bream, *Pagrus major* and its effects on growth, enzyme activity, plasma parameters and apparent nutrient digestibility. The Israeli Journal of Aquaculture-Bamidgeh 66:210-1021

Maralit BA, Ventolero MFH, Maningas MBB, Amar EC, Santos MD, 2014. Subtracted transcriptome profile of tiger shrimp (*Penaeus monodon*) that survived WSSV challenge. Dataset Papers in Science 2014: e807806 (11 pages) (dataset paper)

Marcial HS, Suga K, Kinoshita S, Kaneko G, Hagiwara A, Watabe S. 2014. Molecular cloning and localization of GABAA receptor-associated protein in the rotifer *Brachionus plicatilis*. International Review of Hydrobiology 99:188-197

Narceda A, Montojo UM, Eguia MRR, Sia Su GL. 2014. Paralytic shellfish poisoning toxin accumulation in shellfishes collected from various habitats in Murcielagos bay, Philippines during harmful algal blooms occurrence. Advances in Environmental Biology 8:2262-2265

Orozco ZGA, Sumbing JG, Lebata-Ramos MJH, Watanabe S. 2014. Apparent digestibility coefficient of nutrients from shrimp, mussel, diatom and seaweed by juvenile *Holothuria scabra* Jaeger. Aquaculture Research 45:1153–1163

Primavera JH. 2013. The Agusan Marsh and the Agusan River Basin: The need for science-based development and management. In: Aralar MLC, Borja AS, Palma AL, Mendoza MM, Ocampo PC, Manalili EV, Darvin LC (eds.). LAKECON2011: Building on the Pillars of Integrated Lake Basin Management. (Second National Congress on Philippine Lakes) Summary of Proceedings. Los Baños, Laguna: Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development: Department of Science and Technology; pp 96-101

Romana-Eguia MR, Laron MA. Catacutan MR. 2013. On-farm feed management practices for Nile tilapia (*Oreochromis niloticus*) in the Philippines. In: Hasan MR, New MB (eds.). On-Farm Feeding and Feed Management in Aquaculture. FAO Fisheries and Aquaculture Technical Paper No. 583. Rome: Food and Agriculture Organization; pp. 131-158

Santos BS, Romana-Eguia MRR, Basiao Z, Ikeda M. 2014. Development and characterization of nine novel microsatellite markers for the milkfish *Chanos chanos*. Conservation Genetics Resources (online first) (microsatellite letters)

Sarinas BGS, Gellada LD, Magramo MM, Baria LO, Tirazona DB, Sorio LRD, Tornalejo JA. 2014. Plankton diversity in ballast water of an inter-island passenger-cargo ship calling the Philippine ports. Asian Journal of Biodiversity 5:ajob. v5i1.482

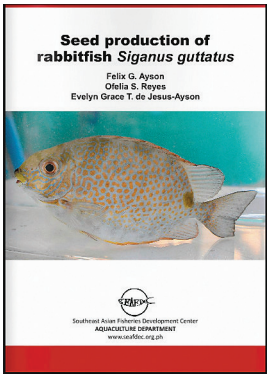
Sarinas BGS, Gellada LD, Torrigue MLT, Sibonga DN, Torrato ES, Malagad JG, Feril JG, Bondoc LAJ, Roncal JCA, Tornalejo JA. 2014. Adsorption and biomass concentration of thraustochytrid *Schizochytrium aggregatum* (Goldstein and Belsky) in bunker C oil. Journal of Environmental Science and Management 17:68-71 (research note)

Seraspe EB, Gabotero S, De la Peña MR, Pahila IG, Amar EC. 2014. Evaluation of dietary freeze-dried *Chaetoceros calcitrans* supplementation to control *Vibrio harveyi* infection on *Penaeus monodon* juvenile. Aquaculture 432:212–216. (short comm.)

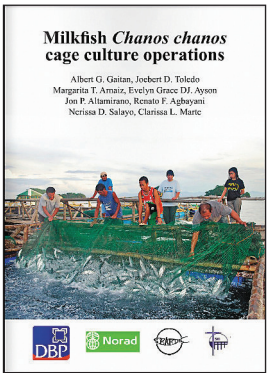
Suyo JB, Prieto-Carolino A, Subade RF. 2013. Reducing and managing disaster risk through coastal resource management: A Philippine case. Asian Fisheries Science 26:198-211

Extension Manuals

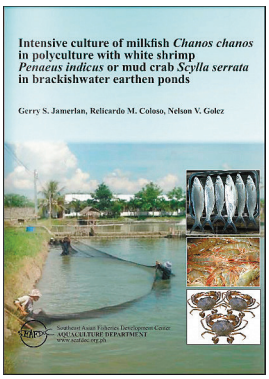
The year 2014 was a productive year for SEAFDEC/AQD in terms of extension manuals. This is in response to the clamor of stakeholders for timely technical information on aquaculture commodities and AQD gladly responded with these science-based guides on the various aspects of culture of traditional and emerging species.



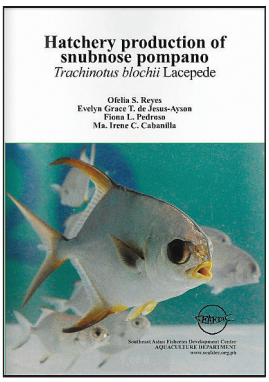
AEM 59 Seed production of rabbitfish *Siganus guttatus* by Felix Ayson, Ofelia Reyes, Evelyn Grace Ayson. A 19-page extension manual describing the biology, site selection, broodstock management, larval rearing and larval food production for rabbitfish



AEM 58 Milkfish *Chanos chanos* cage culture operations by Albert Gaitan, Joebert Toledo, Margarita Arnaiz, Evelyn Grace Ayson, Jon Altamirano, Renato Agbayani, NerissaSalayo, Clarissa Marte. A 39-pages extension manual describing the biology, fingerling production, site selection, cage design and construction, measurement & analysis of water & sediment quality parameters and economic analysis.



AEM 57 Intensive culture of milkfish *Chanos chanos* in polyculture with white shrimp *Penaeus indicus* or mud crab *Scylla serrata* in brackishwater earthen ponds by Gerry Jamerlan, Relicardo Coloso, Nelson Golez. A 29-page extension manual describing the biology, site selection, pond management and harvest & post-harvest of milkfish in intensive polyculture with crab and shrimp in earthen pond.



AEM 56 hatchery production of snubnose pompano *Trachinotus blochii* Lacepede by Ofelia Reyes, Evelyn Grace Ayson, Fiona Pedroso, Ma. Irene Cabanilla. A 26-page extension manual describing the biology, broodstock acquisition & management, larval rearing, harvest & transport and prevention of diseases & parasites in the hatchery production of pompano.

Fairs and Exhibits

In line with SEAFDEC's mandate to improve its visibility and reach out to stakeholders, SEAFDEC/AQD participated in 5 events last year to better inform the general public on AQD's research and extension activities. A total of 1,715 aquaculturists, businessmen, students and government officials visited its booths, signed its guestbook and discussed with AQD resource persons and information specialists on various topics in aquaculture and stock enhancement.

International Workshop on Resource Enhancement and Sustainable Aquaculture (IWRESA); 5-7 March 2014; Iloilo City, Philippines. The SEAFDEC Aquaculture Department together with the Government of Japan held a 3-day international workshop to promote resource enhancement and sustainable aquaculture. See details on page 47.

5th Aquatech: Aquaculture Expo & Convention Philippines 2014; 29-31 May 2014; Dagupan City, Philippines. Aquatech Philippines is an annual event organized to keep the industry updated on new aquaculture developments and technologies, current issues and policies as well to showcase new products and services for aquafarmers. AQD scientists Dr. Rolando Pakingking Jr., Dr. Emilia Quinitio and Dr. Nerissa Salayo gave seminars on the diseases in tilapia, updates on soft-shell crab production and market & development issues in coastal resources management, respectively. Moreover, AQD scientists Dr. Ma. Lourdes Aralar, Dr. Mae Catacutan, Dr. Emilia Quinitio, and Ms. Jocelyn Ladja were among the resource persons during the convention's aquaculture clinic while Dr. Maria Rowena Eguia and Dr. Salayo served as moderators during the technical conference. The AQD Chief Dr. Felix Ayson, Deputy Chief Dr. Takuro Shibuno, Research Division head Dr. Ma. Junemie Hazel Lebata-Ramos, and Dr. Veronica Alava also attended the event.

9th National Shrimp Congress; 2-4 July 2014; Bacolod City. This event had technical sessions on the updates of shrimp aquaculture such as pond water quality and health management, feed development and feeding strategies. Additional topics discussed were on marketing, financing as well as the enforcement of laws relevant to shrimp aquaculture. There were also poster presentations from AQD: "Luminescent vibriosis in *Penaeus vannamei*: First case in the Philippines," "Molecular detection of *Vibrio harveyi* in Asian sea bass, *Lates calcarifer* using dnaJ gene as marker," "Antibacterial activity of extracts from Panay seaweed (*Ulva pertusa* Kjellman)," and "Survey of antibiotics and organochlorine pesticide residues from selected locations in the Philippines." AQD also participated in the event's exhibit which enabled it to reach more aquaculture stakeholders. All these activities, plus the brisk sales of SEAFDEC/AQD's publications helped to disseminate AQD's science-based aquaculture technologies.

AgriLink/FoodLink/AquaLink 2014; 9-11 October 2014; Pasay City, Philippines. SEAFDEC/AQD is a regular participant of the annual AgriLink fair. AQD technical resource persons provided free consultations and discussions with entrepreneurs, students and government personnel. The fair also served as a feedback mechanism for AQD scientists on possible areas of research or commodities to be explored in the future. Dr. Relicardo Coloso and Ms Ma. Rovilla Luhan delivered short lectures on "SEAFDEC premium diet for milkfish" and on "The latest advances in the culture of the seaweed, *Kappaphycus*," respectively. Free flyers and brochures about SEAFDEC/AQD activities were handed out while the sales of the extension manuals amounted to P51,690. The best sellers for 2014 were manuals pertaining to mud crab hatchery and growout, outselling last year's best sellers which were manuals on tilapia.

International Seaweed Conference; 19-21 November 2014; Cebu City, Philippines. The more than 700 participants to this conference are the stakeholders of the seaweed industry that included farmers, processors, exporters, government officials, decision-makers, entrepreneurs, scientists and the academe. International experts presented papers on various topics such as development of seaweed farming techniques and the status and demand of the different seaweed species. The SEAFDEC/AQD delegation consisted of the AQD Chief Dr. Felix G. Ayson, seaweed scientist and researchers Ms. Ma. Rovilla Luhan, Ms Jonalyn Mateo, Mr. Keneth Tibubos and AQD Information Specialists Mr. Isidro Tendencia and Mr. Jesus Manolo Almendras.

[L-R] AQD scientists Dr. Emilia Quinitio, Dr. Rolando Pakingking Jr. and Dr. Nerissa Salayo speak during AQUATECH's technical conference

AQD's Dr. Relicardo Coloso (L) discussing the AQD milkfish premium diet with Dr. Patrick White (M) and Dr.Tom Shipton (R) during the AgriLink fair

The 9th Shrimp Congress booth manned by Ms Rossea Ledesma and Ms Joessyl Marie de la Cruz

SEAFDEC/AQD booth during the International Seaweed Conference showing AQD's seaweed scientist Ms Maria Rovilla Luhan; AQD's Chief, Dr. Felix Ayson; the Governor of the Province of Palawan, Hon. Jose Alvarez and the Mayor of Tudela, Mr. Erwin Yu.

Resource Enhancement in the ASEAN Region

To promote and augment regional initiatives on resource enhancement and sustainable aquaculture practices, and to contribute to poverty alleviation, livelihood and food security, SEAFDEC Aquaculture Department together with the Government of Japan held a 3-day international workshop on resource enhancement and sustainable aquaculture practices in Southeast Asia on 5-7 March 2014 at Iloilo City, Philippines.

Aquaculture is undoubtedly a practical way to reduce fishing pressure on wild aquatic species and is an effective measure to fulfill man's demand for food fish without harming wild resources. Release programs, that actively repopulate local stocks, ascribe to the development of seed production technologies in aquaculture. Moreover, aquaculture is addressing the issues of food security and widespread poverty in the Southeast Asian region. However, broodstock and fry of not a few aquaculture species, particularly high value species, still depend on wild resources. Hence, fishing pressure on these species has seriously affected the sustainability of these fishery resources.

Endeavors toward resource enhancement and sustainable aquaculture practices are complement efforts in securing livelihood and decreasing poverty in Southeast Asia. With regard to resource enhancement, stocking through release programs together with appropriate fishing regulations are recognized as effective means to restore the population of overexploited aquatic species. Although an array of seed production technologies and hatchery practices have been developed for numerous species in several countries, thus far, practical information and skills which could be utilized for stock enhancement are still lacking in most Southeast Asian countries. Similarly, promotion of sustainable and environment-friendly aquaculture practices still need to be pursued through the development of region-oriented technologies and knowledge.

For most ASEAN countries in various stages of implementing resource enhancement programs, the best lessons from IWRESA presentations and discussions are: (1) on-site hatcheries may work best as these can ensure the use of local breeders for local waters; and (2) improving stock management and human governance to ensure that resource enhancement benefits both communities and the environment.

SEAFDEC/AQD's own efforts in resource enhancement prioritize the seahorses *Hippocampus* spp., tropical abalones *Haliotis* spp., the mudcrab *Scylla* sp., and the sandfish *Holothuria* sp. Release strategies of these commodities are still being studied by AQD scientists.

The details of the workshop discussion are described in the workshop report together with the book of abstracts, and are available at the SEAFDEC/AQD homepage: http://www.seafdec.org.ph/2014/resource-enhancement-work/.

(L-R) Plenary speaker Dr. Hitoshi Araki of Hokkaido University, workshop organizing committee chair and Deputy Chief Dr. Teruo Azuma, SEAFDEC Deputy Secretary General Mr. Hajime Kawamura, and Dr. Koichi Okuzawa of Japan Fisheries Research Agency

Regional Technical Consultation on *Fish Meal Substitutes in Aquaculture Feed Formulation*

The Regional Technical Consultation (RTC) on "*Development and Use of Alternative Dietary Ingredients or Fish Meal Substitutes in Aquaculture Feed Formulation*" was convened in Nay Pyi Taw, Myanmar on 9-11 December 2014.

Marine resources such as fish meal and fish oil are major feed components of aquaculture feed and are under increasing exploitation due to the increased volume of feed production in the ASEAN region. The efficient use of aquaculture feeds and continued research for developing suitable alternative protein sources to reduce dependence on fish meal and other fish-based products were adopted by the ASEAN Member States in June 2011 as part of the Plan of Action on Sustainable Fisheries for Food Security towards 2020. To pursue this recommendation, the Aquaculture Department (AQD) and the Secretariat of SEAFDEC, and the Government of Myanmar, through funds provided by the Japanese Trust Fund (JTF) and ASEAN-Japan Solidarity Fund, convened the meeting that brought together over 50 participants from SEAFDEC Member Countries, technical experts on aquaculture feed, officers from the ASEAN Foundation, from SEAFDEC and the FAO Regional Office for Asia and the Pacific (FAO/RAP), and representatives from feed companies. Country representatives gave updates on utilization of fish by-catch, fish meal and plant protein sources in the commercial production of aquaculture feed as well as the feeding practices that can improve feed efficiency. The participants reviewed the status of developing alternative dietary ingredients for aquaculture feeds of the Member Countries including constraints and recommendations on the use of alternative protein sources and the existing regional policies and initiatives that limit the use of feed ingredients of marine origin. Invited speakers, including the feed and nutrition experts from AQD, presented the technological developments on the use of plants and other alternative feed ingredients as protein sources. The highlight of the meeting was a workshop session where participants, in working groups, identified the key issues under the thematic areas, the research gaps and recommendations for the trophic groupings of herbivores, carnivores and omnivores. The session culminated with a plenary presentation on the regional policy recommendations and immediate actions needed for the development and use of alternative dietary ingredients in aquaculture feed formulation. Finally, the ASEAN Member States, organizations and other partners in the region renewed their commitment to enhance collaborative efforts and strengthen partnerships in reducing dependence of aquaculture on feed ingredients of marine origin.

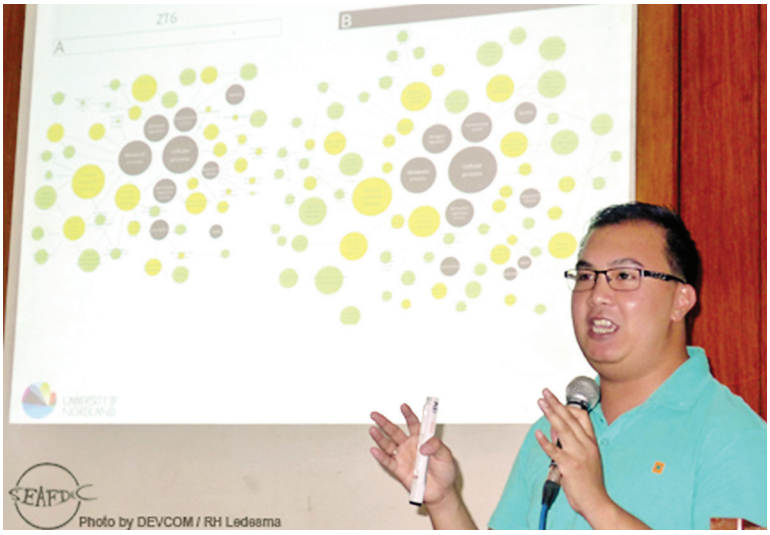
Participants of the ASEAN-SEAFDEC RTC on Development and Use of Alternative Dietary Ingredients or Fish Meal Substitutes in Aquaculture Feed Formulation

VISITORS

SEAFDEC/AQD welcomed 21,099 visitors to its four stations, the bulk of which, around 63%, visited the Igang Mariculture Park. While most of these visitors came from the private sector, some 33% came from the academe and a few of them gave seminars about their studies.

Dr. Annabelle Villalobos a DOST Balik-Scientist and Johnson Consultant, BioPharmaceuticals R&D (USA), received a certificate of appreciation from AQD Chief Dr. Felix Ayson for her seminar on "Pharmaceuticals, from lab to market" on 31 July 2014.

Mr. Matthew Tan, Chief Technology Officer of Oceanus Group Ltd. gave a seminar on "The Industrialization of the Abalone Industry in China by the Oceanus Group" and "Operations of a Mega-Sized Abalone Farm" on 19 May 2014.



Dr. Carlo Lazado of University of Nordland (UiN), Norway, discussed his dissertation on "Molecular basis of daily rhythmicity in fast skeletal muscle of Atlantic cod *(Gadus morhua)*" on 24 October 2014.

Dr. Casiano Choresca Jr. a faculty of the graduate school at the Iloilo State College of Fisheries in Barotac Nuevo, Iloilo, talked about his study on "Transfection of the red fluorescent protein gene in the goldfish caudal fin-derived somatic fibroblasts cells" on 9 January 2014.

Staff Development

In 2014, SEAFDEC/AQD supported the attendance of its research and information personnel to various meetings and conferences where they presented papers and/or posters or learned from the training courses they attended.

Training Workshop on Implementing QA/QC in Analytical Chemistry Laboratories in Preparation for ASEAN 2015 organized by Integrated Chemist of the Philippines in cooperation with the PNP Crime Laboratory on 17-18 February 2014 at Camp Crame, EDSA, Quezon City, Philippines.

Philippine Studies Conference in Japan (PSCJ) on 27 February - 02 March 2014 at the University of Kyoto, Japan.

IOC WestPac 9the Scientific Conference, 22-25 April 2014 UNESCO Intergovernmental Oceanographic Commission, Western Pacific SubCommission in Nha Trang, Viet Nam (trip was cancelled due to visa problems)

World Aquaculture Society Meeting, Status of Aquatic Animal Health Care in Small-Scale Freshwater Aquaculture in Southeast Asia on 07-12 June 2014 in Adelaide, South Australia, Australia.

Special Libraries Association Annual Conference: Mapping the Distribution, Use and Visibility of Publications and Library Services: Library Initiatives Beyond Boarders, 08-10 June 2014 in Vancouver, Canada.

Hokkaido University, to engage in studies in seed production and release for stock enhancement of seahorses under the framework of JSPS (Japan Society for Promoton of Science) - RONPAKU (Dissertation PhD) Program, 30 June-31 July 2014 at Hokkaido University, Japan.

International Institute for Fisheries Economics and Trade (IIFET) Conference on 07-11 July 2014 at Queensland Univeyrsity of Technology, Brisbane, Australia.

40th Conference of the International Association of Aquatic and Marine Science Libraries & Information Centers (IAMSLIC) Resource Sharing Committee on 14-18 September 2014 at the Secretariat of the Pacific Community (SPC) in Noumea, New Caledonia.

9th Shrimp Congress organized by BFAR, PHILSHRIMP/NPPC, SEAFDEC, DOST-6 & DTI on 02-04 July 2014 at the Bacolod Pavillon Hotel, Bacolod City.

Training Course on Microbiological Analysis of Food on 04-08 August 2014 at the University of the Philippines, Diliman, Natural Sciences Research Institute, Quezon City, Philippines.

15th World Lake Conference: Mirrors of the Earth- Balancing Ecosystem Integrity and Human Well-being. 31 August-5 September 2014, Perugia, Italy.

Short-term Training sponsored by GOJ (arranged by the Deputy Chief, Dr. T. Shibuno) on 31 August - 12 September 2014 in Japan.

Training on Radiation Safety Course on Sealed Sources in Industrial Devices (RSC-ID) on 08-12 September 2014 at he Philippine Nuclear Research Institute, Quezon City, Philippines.

9th Symposium on Diseases in Asian Aquaculture (DAA9) Fish Health Section of Asian Fisheries Society on 24-28 November 2014 at the Rex Hotel, Ho Chi Mihn, Viet Nam.

Mr. Daryl Superio of AQD Library (leftmost) together with the representatives of ASFA board and library partners during the IAMSLIC 40th conference in Noumea, New Caledonia.

SEAFDEC/AQD scientist Dr. Rolando Pakingking Jr presenting his study on bacterial microbiota of tilapia

Sci-Art AquaWeek Year 14

As part of SEAFDEC/AQD's 41st Anniversary Celebration, AQD Museum FishWorld held its 14th Sci-Art AquaWeek from28 July to 01 August 2014.

The Aquaculture Department of SEAFDEC advances responsible aquaculture and supports sustainable development in the Philippines and Southeast Asia. SEAFDEC FishWorld is dedicated to science and environment education of the general public - children, adults, students, teachers, researchers, public officials, tourists and local residents particularly about aquatic ecosystems, biodiversity, aquaculture, fisheries, and marine conservation.

Sci-Art AquaWeek seeks to build understanding and appreciation among school children and teachers in Panay of the research and development work of SEAFDEC/AQD and the relations between aquatic biodiversity, aquaculture, fisheries, human nutrition, and economic advancement. Such understanding is then expressed through contests with various outputs. Sci-Art AquaWeek 2014 focuses on (1) aquaculture and its importance to food security, and (2) the Philippines being the center of the center of marine biodiversity and how Filipinos should know more about this natural heritage. Five contests are open to all high schools, and four contests to all elementary schools.

These are: Biodiversity Lab: "*Fish diversity around Panay and Guimaras*"; Biodiversity Workshop and Quiz: "*The Philippines is the center of mollusk diversity*"; Painting: "*The Philippines is the center of marine biodiversity*"; Powerpoint Seminar: "*Aquaculture versus fisheries*"; Bring, Show and Tell (by teachers): "*Farmed seafood for good health*"; Nutrition and Aquaculture Quiz; Write and Draw a Booklet: "*Paglaki ko, I want to be a fish farmer*"; Aquarium Quiz: "*Lantawa!*" and Seafood Skills Olympics: "*Preparing and serving sutokil.*"

Upper left: FishWorld curator Dr. Teodora Bagarinao (right) with some of the students and teachers who joined the Sci-Art contests.

Left: Dr. Takuro Shibuno (AQD's Deputy Chief, 2nd from left) and Dr. Evelyn Grace D. Ayson (AQD's TID Head, 4th from left) with the teachers and students during **Seafood Skills Olympics: "*Preparing and serving sutokil.*"**

Above: The winning poster during the on-the-spot painting contest with the theme **"*The Philippines is the center of marine biodiversity*"**

2014 production from ponds and cages...

SEAFDEC/AQD produced about 20 tons market-sized commodities from research activities. The bulk of the production was from studies on milkfish of about 15 tons.

Species	Production (Kg)		Total
	Ponds	Cages	
Milkfish	9,730	4,900	14,630
Pompano	17	2,696	2,713
Seabass	328		328
Rabbitfish	1,036		1,036
Abalone		8	8
Mangrove crab	575		575
Tilapia		10	10
Giant freshwater prawn		6	6
Total	**11,688**	**7,620**	**19,308**

2014 Hatchery production of marine and freshwater species at SEAFDEC/AQD

Species	Production (pcs)	
	larvae	fry
Pompano	403,246	101,400
Grouper	42,630,066	37,702
Seabass	12,310,504	554,848
Rabbitfish	11,014,920	44,619
Milkfish	91,271,427	7,610,308
Snapper	7,032,800	21,028
Tilapia		41,174
Bighead Carp		490,475
	postlarvae	**juveniles**
Giant Freshwater Prawn	161,850	2,460

Feedmill production

SEAFDEC/AQD feed mill produced more than 65,000 kilos of various feeds with 27% going to the private sector (most for milkfish) and the rest to AQD studies

Aquafeed Produced at the Feed Mill	
Feed Type	**Volume (Kg)**
Milkfish	
Broodstock Grow-out Larval	21,705 27,555 560
Pompano	897
Seabass	1,880
Grouper	1,059
Siganid	4,572
Tilapia	331
Mangrove crab	4,475
Shrimp	2,108
Abalone	70
T O T A L	65,212

Fish health diagnostic services...

At AQD's fish health laboratory, a total of 474 diagnostic cases were examined consisting of shrimp (approx 55%); fish (10%); crab (17%); mollusks (6.3%); water (8%); and other samples such as annelids, oysters, seaweeds and white mouse (3.6%). The eight viral diseases diagnosed by PCR were: WSSV, 114 of 1,119 samples positive (10.2%); IHHNV, 16 of 476 samples positive(3.4%); IMNV , 0 of 486 samples positive; TSV, 0 of 446 samples positive; YHV/GAV, 0 of 387 samples positive; PvNV, 0 of 4 samples positive; VNN, 20 of 51 samples positive (39.2%); RSIV, 0 of 22 samples positive AQD also accepted samples for bacterial count (412 samples), bacterial identification (43), presence of parasites (1 of 8 samples positive or 12.5%), and histological samples for research or disease diagnosis (2,722 slides).

...and LFAAT AQD's Laboratory Facilities for Advanced Aquaculture Technologies (LFAAT or the Biotech Lab), accepted for analysis a total of 3,938 samples (or 6,650 determinations) the bulk of which came from AQD research studies and a few from the private sector. Some 1,270 of these samples were for proximate analyses for crude protein, crude fat, crude fiber, calcium, phosphorus, moisture, and ash; fatty acid profile; 1,849 for water analyses for pH, alkalinity, ammonia-N, ammonium-N, chlorophyll, dissolved oxygen, nitrite, nitrate, phosphate, sulfide, total hardness, total suspended solids, and pesticide residues; 515 for soil analyses for pH, organic matter, available Fe / S / P, and CNS;

53 microbiological analyses for APC, *Salmonella, Shigella, Staphylococcus aureus*, coliform, *Escherichia coli, Vibrio*, yeast & mold, and water potability; and 12 for scanning & transmission electron microscopy. The LFAAT staff were also involved in the conduct of training and seminars and have started activities for AQD's ISO accreditation.

Larval food production

To support the research and production activities of AQD, the larval food laboratory served 112,603 liters of microalgal feeds or starters and 8 kg (wet weight) of *Artemia* biomass. The laboratory also sold 2,297 liters of microalgal or rotifer starters to 226 clients comprising of the private sector (local, 59.7%; foreign, 9.7%); academe (26.1%); and government institutions (4.4%). Earnings amounted to Php 330,156.

About 14 kgs of concentrated microalgal paste was also produced. Thirty tube cultures of *C. calcitrans*; twenty eight tubes of *Thalassiosira* sp.; one of *Tetraselmis tetrahele* and ten of *C. sorokiniana* were sold. Pre mixed fertilizers and culture media were also sold: 163 liters of TMRL, 87.55 liters of F medium and 28.45 liters of Conwy medium.

Of the 2,297 liters of liquid starters sold the following phytoplankton species make up the percentage at right:

Species	percent
Skeletonema tropicum	29.00
Nanochlorum sp.	14.00
Chaetoceros calcitrans	19.00
Brachionus rotundiformis	3.20
Tetraselmis tetrathele	19.10
Chlorella sorokiniana	7.00
Navicula ramossisima	1.10
Thalassiosira sp.	1.20
Isochrysis galbana	3.00
Amphora sp.	0.43
Rhodomonas sp.	0.01
Chaetoceros muelleri	0.15
Chaetoceros sp.	0.07
Nanochloropsis sp.	0.44
Chlorella sp.	0.91

Stock cultures of microalgae kept in test tubes at AQD's larval food production laboratory

Statement of Financial Position

STATEMENT OF FINANCIAL POSITION (US Dollar '000)		
	As of Dec. 31, 2014	*As of Dec. 31, 2013*
ASSETS		
CURRENT ASSETS		
Cash & cash equivalents	2,896	2,270
Accounts Receivables	664	268
Materials and Supplies	53	72
Other current assets	2	1
Total Current Assets	3,615	2,611
NON-CURRENT ASSETS		
Cash investments	74	218
Other non-current assets	377	331
Total Non-current Assets	451	549
TOTAL ASSETS	4,066	3,160
LIABILITIES		
CURRENT LIABILITIES		
Accounts Payable	1,854	405
Funds Held-in-Trust	369	324
TOTAL LIABILITIES	2,223	729
NET ASSETS		
Designated	1,843	2,417
Undesignated	-	-
Unrealized Gain on AFS Financial Assets		14
TOTAL NET ASSETS	1,843	2,431
TOTAL LIABILITIES AND NET ASSETS	4,066	3,160

STATEMENT OF REVENUES & EXPENDITURES (US Dollar '000)		
	Period ending Dec. 31, 2014	Period ending Dec. 31, 2013
REVENUES		
Contributions / Grants	5,238	4,739
Other Income	634	595
TOTAL REVENUE	5,872	5,334
EXPENDITURES		
Research Programs	3,875	3,342
Training Programs	163	301
Information Programs	373	123
General Administrative & Non-Project Expenses	2,018	1,162
TOTAL EXPENDITURES	6,429	4,928
BALANCE	(557)	406

AQD personnel distribution

As of 31 December 2014, AQD had a total personnel complement of **179** (regular employees, 100; fixed-term employees, 79)

AQD officers in 2014

AQD Chief	Dr. Felix Ayson
Deputy Chief	Dr. Takuro Shibuno
Head, RD	Dr. Ma. Junemie Hazel Lebata-Ramos
Head, TVDD	Dr. Mae Catacutan
Head, TID	Dr. Evelyn Grace Ayson
Head, AFD	Ms. Kaylin Corre
Head, BFS	Dr. Frolan Aya
Head, DBS	Dr. Emilia Quinitio
Head, IMS	Mr. Mateo Paquito Yap
Head, Manila Office	Dr. Ma. Rowena Eguia
Program leaders	
Producing quality seed for sustainable aquaculture	Dr. Ma. Rowena Eguia
Promoting healthy & wholesome aquaculture	Dr. Myrna Teruel and Dr. Rolando Pakingking
Maintaining environmental integrity through responsible aquaculture	Dr. Ma. Lourdes Aralar
Adapting to climate change	Dr. Fiona Pedroso
Meeting socio-economic challenges in aquaculture	Dr. Nerissa Salayo
Regional programs	Dr. Takuro Shibuno
Section heads	
[RD]	
Breeding and seed production	Dr. Fe Dolores Estepa
Fish health	Dr. Edgar Amar
Nutrition and feed development	Dr. Mae Catacutan
Farming systems and ecology	Ms Ma. Rovilla Luhan
Socioeconomics	Dr. Nerissa Salayo
[TVDD]	
Technology verification	Dr. Myrna Teruel
Demonstration & packaging	Dr. Veronica Alava
[TID]	
Training	Dr. Evelyn Grace Ayson
Development communication	Mr. Jesus Manolo Almendras
Library and data banking services	Mr. Stephen Alayon
[AFD]	
Engineering	Engr. Arnel E. Gelvero
Human resources management	Atty. Gina Gomez/ Ms Nira Grace Llona
Budget-cashiering	Ms Jiji Rillo
Accounting	Ms Marivic Guevara/ Ms Jasmine Gelvero

RD, Research Division
TVDD, Technology Verification & Demonstration Division
TID, Training & Information Division
AFD, Administration & Finance Division
BFS, Binangonan Freshwater Station
DBS, Dumangas Brackishwater Station
IMS, Igang Marine Station

www.seafdec.org.ph

The Southeast Asian Fisheries Development Center (SEAFDEC) is a regional treaty organization established in December 1967 to promote fisheries development in the region. The member-countries are Brunei Darussalam, Malaysia, Myanmar, Philippines, Singapore, Thailand and Vietnam. The policy-making body of SEAFDEC is the Council of Directors, made up of representatives of member countries. SEAFDEC has five technical departments that focus on different aspects of fisheries development:

- **Training Department** (TD) in Samut Prakan, Thailand (1967) for training in marine capture fisheries
- **Marine Fisheries Research Department** (MFRD) in Singapore (1967) for postharvest technologies
- **Aquaculture Department** (AQD) in Tigbauan, Iloilo, Philippines (1973) for aquaculture research and development
- **Marine Fishery Resources Development & Management Department** (MFRDMD) in Kuala Terengganu, Malaysia (1992) for the development and management of fishery resources in the exclusive economic zones of SEAFDEC member countries
- **Inland Fishery Resources Development and Management Department** (IFRDMD) in Palembang, Indonesia (2014) for sustainable development and management of inland capture fisheries in the Southeast Asian region

AQD is mandated to:

- Conduct scientific research to generate aquaculture technologies appropriate for Southeast Asia
- Develop managerial, technical and skilled manpower for the aquaculture sector
- Produce, disseminate and exchange aquaculture information.

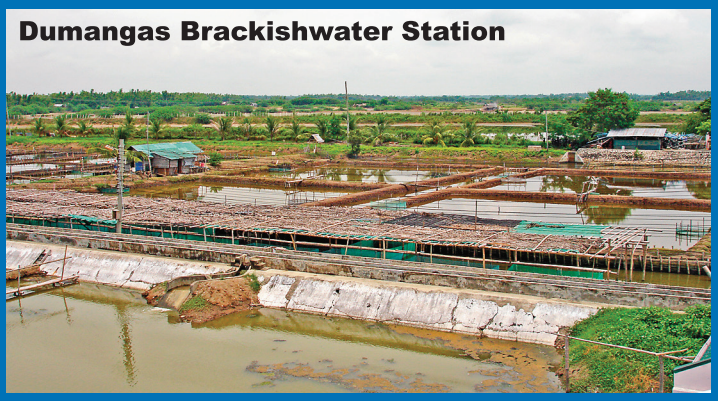
AQD maintains four stations: Tigbauan Main Station and Dumangas Brackiswater Station in Iloilo province; Igang Marine Station in Guimaras province; and Binangonan Freshwater Station in Rizal province. AQD also has a Manila Office in Quezon City.

Email: aqdchief@seafdec.org.ph
Tel. (63 33) 330 7000 or 511 9170 *Fax* (63 33) 330 7031

Tigbauan Main Station

Binangonan Freshwater Station

Dumangas Brackishwater Station



Igang Marine Station